THE PHYSIOLOGY AND PHARMACOLOGY OF SOME CELLULOSE DERIVATIVES

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A. INTRODUCTION

Definition of macromolecules

Increasing knowledge in physics and chemistry has stimulated research on high polymers, or to use another term, on macromolecules and on their applications in various fields. The definition of macromolecules given in 1922 by Staudinger and Fritschi states that a macromolecule is a compound with more than 1,500 atoms and with a molecular weight exceeding 10,000; dissolved it shows colloidal properties and cannot be dialyzed. Before this statement made by Staudinger and Fritschi "supramolecular" structures were given various names as for instance "bioblasts". They were thought to be built of particles "which cannot be seen under the microscope and whose properties such as assimilation, growth, and propagation are strictly different from the atoms and molecules dealt with in physics and chemistry" (Hertwig, 1923). Although it was agreed in the different research centres of physical chemistry that the old concept of "bioblasts" or "biophores" should be abandoned, biologists as recently as 1923 were referring to and defining various biological materials, which were neither inorganic nor living, as "bioblasts". This is the more surprising as, before this time, Emil Fischer (1906) thought that proteins were composed of small molecules,

and even as far back as 1877 Naegeli and Schwendener postulated a supramolecular grouping of living material; this was believed to be formed by the linkage of molecules and was described as a micellar aggregate. Meyer and Mark (1928) stated that micellar aggregates are bundles of main-valency chains; these chains were believed to be the units from which substances of living matter were made (Meyer and Mark, 1940). The theory of micellar aggregates was accepted until the macromolecular structure of compounds was demonstrated by Staudinger and Lüthy (1925), Haworth and Peat (1926), Consden, Gordon and Martin (1947) and others, and the synthetically produced substances were compared with natural products such as hydrocarbons or, to a lesser extent, proteins. Until recently scientists were unable to produce macromolecules synthetically. The importance of the synthesis of high polymers lies in the fact that it may greatly facilitate the understanding of macromolecular structures in living matter.

One of the first substances used was polyoxymethylene (Staudinger, 1925). A polymer homologous series of polyoxymethylene diacetates can be achieved by gradual degradation with acetic anhydride. Such a series is called polymer homologous because it comprises chains of molecules of uniform structure but of different size.

Fig. 1

Formulae of polyoxymethylene derivatives

$$OH - CH_2 - O - (CH_2 - O)_n - CH_2 - OH$$

Polyoxymethylenedihydrate

n = 10 to 150

$$CH_3O - CH_2 - O - (CH_2 - O)_n - CH_2 - OCH_3$$

Polyoxymethylenedimethyl ether

n = 10 to 150

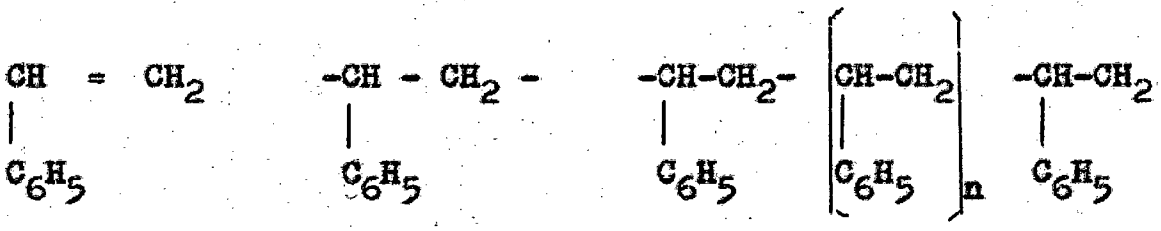
The size of such a macromolecule is expressed by the degree of polymerisation, i.e. the number of units which forms the molecule.

Another method of obtaining polymer homologous series is the addition of methanol and sulphuric acid to polyoxymethylene which leads to the formation of series or chains of polyoxymethylenedimethyl ethers (Staudinger, 1925). There is a similarity between such a synthetic product and, for instance, cellulose and, as a substance with similar physical and chemical properties of cellulose, a series of polyoxymethylene has been used. The degradation of polyoxymethylene depends on its solubility. Polyoxymethylenedihydrate for instance possesses an OH end-group and NaOH can attack the molecule there, whereas the CH_3 end-group in polyoxymethylenedimethyl ethers prevents the breaking up of the molecule and thus its solubility in an alkaline solvent (Fig. 1). A small number of end-groups, often less than 1% of the total molecule, may thus influence the chemical reaction of such a macromolecule very markedly.

Another compound which has been used as a model for typical colloidal substances, especially hydrocarbons such as rubber, is polystyrol. The polymer homologous series of polystyrol reacts in many ways similarly to isoprene which forms the basic molecule of synthetic

Fig. 2

Formula of polystyrol



Styrol Unit of Chain Macroradical

n = 20 - 10,000

rubber and resembles it in its ability to form highly viscous solutions and in its elastic properties (Staudinger, Geiger and Huber, 1929; Staudinger and Frost, 1935; Staudinger, Brunner, Frey, Garbsch, Signer and Wehrli, 1929). The formula of polystyrol is given in Fig. 2.

Properties of macromolecules.

There are many physico-chemical properties which distinguish high polymer substances from the low polymer form. Some of the more important are listed below.

(1) Owing to the chain-forming character of carbon, oxygen, and nitrogen, basic molecular structures consist mainly of these atoms. On the other hand small molecules can be composed of practically any atoms.

(2) Macromolecules can possess different chemical properties without changing their basic structure. Chemically speaking, the macroradical does not change but its end-group may change. (This is of great importance because macromolecules are in fact the skeleton of living matter; it is the cellulose structure which gives the cell of a plant its stability and polypeptides which build up the various cells of an animal.

(5) The pH, solubility, polarity, permeability, and density of a substance may change without necessarily

involving a change in its basic high polymer structure.

(4) A macromolecule allows an infinitely greater variation in the composition of its atoms than does a small molecule. It follows that substitutes can more successfully be linked on to a macromolecule than on to small molecules.

(5) The different physical stages of high polymer substances resemble those of protoplasm whereas low molecular forms do not.

(6) Macroradicals have physical properties ranging from a low viscosity to a jelly-like formation. There can be a gradual change from the one form to the other. In protoplasm, the same basic constellation of amino acids can be used to form such diverse molecular structures as blood proteins, or proteins of horny, elastic, or connective tissue. The living substance makes use of this property of high polymers occurring in different forms and reacting in various ways without changing its basic structure. Small molecular compounds do not show these intermediate stages between a liquid and a solid phase and cannot be used to form the matrix of living matter.

(7) Solutions of macromolecules are colloidal whereas small molecular compounds are not. The colloidal properties of a macromolecular solution (e.g. its

viscosity) can be used to calculate the molecular weight; small molecules, not possessing colloidal properties, cannot be used for viscosimetry.

(8) The viscosity of macromolecular solutions depends on the concentration as well as on the shape of the molecule. A solution of glycogen, spherical in shape, shows a much lower viscosity than one containing the same amount of cellulose derivatives, which are linear in shape; this again does not apply to small molecular compounds.

(9) All macromolecular solutions are polymolecular. They are mixtures of molecules of different size but of uniform structure, whereas in small molecular solution the molecules are usually uniform in size and shape whether they appear in a solid, liquid, or gaseous form.

The use of macromolecules in medicine

High polymers were first used as colloidal substances in 1897 in order to increase the coagulability of blood after haemorrhage. They used gelatin for their purpose, and gelatin was used again extensively in the 1914-1918 war as a blood volume expander (Hogan, 1915) but, since shock sometimes followed the infusion, the use of gelatin preparations was discontinued. In spite of many reports against gelatin preparations, the Extra Pharma-

copoeia (Martindale, 1952) still listed gelatin as a blood volume expander. Another property of gelatin, namely its diuretic action, was applied in medicine. After the infusion of 10% gelatin solution into patients suffering from ascites and nephrosis it was found that their condition improved (Scott and Ryan, 1951). Gelatin has also been used as part of the treatment for undernourished patients (Koop, Riegel, Grigger and Barnes, 1947).

Polyvinylpyrrolidone, oxypolygelatin, and dextran are other high polymer substances used with varying success as blood volume expanders. The most obviously undesirable effects of macromolecules injected as blood volume expanders are:

(1) Incomplete breakdown of the high polymer substance.

(2) Storage and tissue irritability

(3) Allergic reactions (Kabat and Berg, 1953; Glynn, Holborow and Johnson, 1954).

After injection of carboxymethylcellulose the growth of benign giant cell tumours was observed (Werthemann and Vischer, 1951) and the Kupffer cells of the liver showed a foamy cytoplasm, while the reticulum cells of the spleen contained many vacuoles (Narat, Casella and Cangelosi, 1952).

High polymer compounds such as methylcellulose are

used in laxatives, e.g. "Tylose" and "Methocel" (Schultz, 1949). The mucilaginous properties of these colloid substances led to their use in cases of duodenal ulcer (Brick, 1949; Hufford, 1951) and greater relief has been reported than with the use of antacid drugs as stated in the Extra Pharmacopoeia (Martindale, 1952, 1958). The stabilizing or emulsifying action of high polymer substances and their use as carriers of potent, quick-acting drugs, led to the production of sorbitan derivatives. Examples of these are polyethylene-glycol, or polyethylene, commercially known as Polysorbas or Carbowax.

Synthetic or semi-synthetic macromolecules have been introduced successfully as anticoagulants. Xylan-sulphuric acid ester (Husemann, Kaulla and Kapesser, 1947), polyanhydromannuronic acid (Seifter, Begany, 1948), as well as dextran sulphate (Ricketts, 1952, 1954; Ricketts and Walton, 1953) show a chemical configuration similar to the chondroitin-sulphuric acid ester, known as heparin. Heparin is very quickly eliminated by the body, while the sulphuric acid ester of xylan may be stored in the body for as long as 14 days (Hoffmann, Husemann, Lötterle, Wiedersheim and Hertlein, 1953). The degree of polymerisation of this ester is the factor which influences its rate of excretion from the body.

Experiments with xylan-sulphuric acid ester-^{35}S showed that the smaller the molecule the greater the rate of excretion of ^{35}S in the urine (Husemann, Hoffmann, Lötterle and Wiedersheim, 1952). It is interesting to note that not only xylan but also cellulose substituted with SO_3 shows anticoagulant properties, but the higher the degree of polymerisation of the product the greater its toxicity (Wiedersheim, Hertlein, Husemann and Lötterle, 1953).

The ability of high polymer substances to combine with the active principles in therapeutic drugs and to allow their gradual release into the blood stream is of toxicological and therapeutic value (Pedersen and Tonnesen, 1950). For example, the toxin-binding properties of polyvinylpyrrolidone led to its introduction into tetanus therapy (Schubert, 1948) and it has been shown that tetanus toxin linked to starch and other polymers of high molecular weight does not cause death in mice injected with 10 times the lethal dose of the uncombined toxin (Hummel, Hertlein and Zöllner, 1955; Wiedersheim, unpublished).

However, as experimentally induced and human tetanus differ in character, it is not advisable to draw conclusions from encouraging findings in laboratory experiments on animals. For many years scientists have tried

to replace bone by celluloid, and neuro- and plastic surgeons experimented in this field with varying success. Only after macromolecular chemistry advanced sufficiently (from 1940 onwards) were surgeons able to make use of stable, hard, and transparent materials such as methylacrylate. Methylacrylate, unlike celluloid, does not show any shadow when used in X-ray photography.

Another high polymer substance is oxidized cellulose, and its derivatives which are used in surgery to combat haemorrhages. It should be noted that these substances could not be applied as permanent surface dressings since they inhibit the formation of epithelium; polythene sprays used as plastic dressings show the same disadvantage. In the field of thoracic surgery polythene spheres have been used with success as thorax plombages. As chemists have been dealing with macromolecules for only a comparatively short time, it is surprising how many macromolecules and derivatives of macromolecules are already in use.

Bioblasts, protomeres, or biophores, believed by biologists of the last century to be living units, were found to be high polymer substances and many of their physico-chemical properties have been elucidated. This is very encouraging as the variation of compounds due

Table 1

Isomers of hydrocarbons

Hydrocarbons	Molecular weight	No. of isomers
C_4H_{10}	58	2 Butane
$C_{10}H_{22}$	142	75 Decosane
$C_{20}H_{42}$	282	366, 319 Eicosane
$C_{30}H_{62}$	422	4×10^9 Triacontane
$C_{40}H_{82}$	562	6×10^{13} Tetracontane

Table 2

Isomers of amino acids

Amino acids	Molecular weight	No. of isomers
3	360	6 Tripeptides
6	760	720 Hexapeptides
10	1200	3×10^6 Decapeptides
20	2400	2×10^8 Eicosapeptides

to isomerism is already considerable, even in the range of macromolecules with a low degree of polymerisation. In Tables 1 and 2 the possible isomers of hydrocarbons and amino acids of relatively low molecular weight are given (Fieser and Fieser, 1956).

The number of possible combinations of living matter having much higher molecular weights than single hydrocarbons or amino acids, are unlimited. For instance in a protein with a molecular weight of 120,000 consisting of 20 different amino acids with about 1,000 amino acid units, the number of possible isomers is approximately 10^{1278} (Fedorn, 1950). All the water molecules in 325 million cubic miles, approximately the volume of all the oceans of the world, give a figure of 4×10^{46} only.

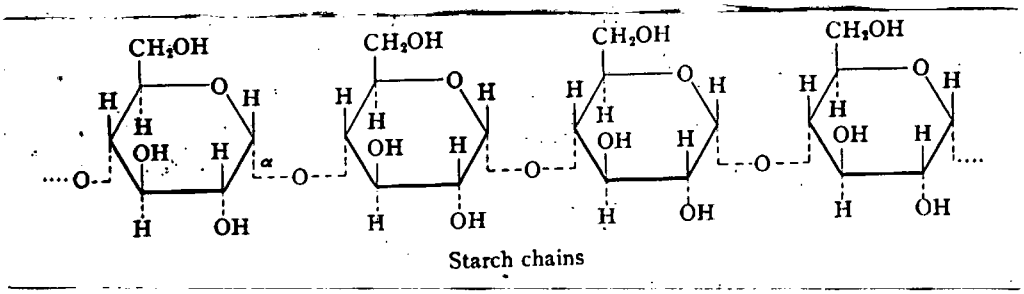


Fig.4 Basic unit and structure of branched high polymers used to study their biological activity.

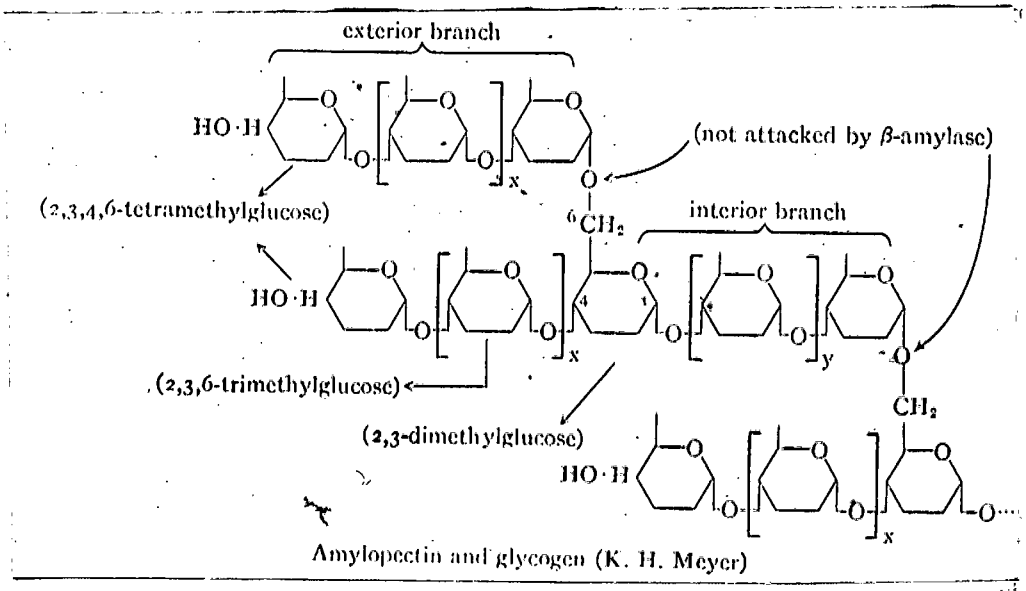


Fig.5 Glycogen and Amylopectin molecules are similar,(Meyer and Mark,1940;Manners,1957).The length of a unit chain of amylopectin varies between 17 and 26 glucose units,depending on the type of starch investigated.(Greenwood and Robertson, 1954;Brown,Halsall,Hirst and Jones,1948;Hassif and McCready, 1943).Amylose,however,consits of linear units as shown in Fig.4, (on average 150-200 glucose units per molecule).

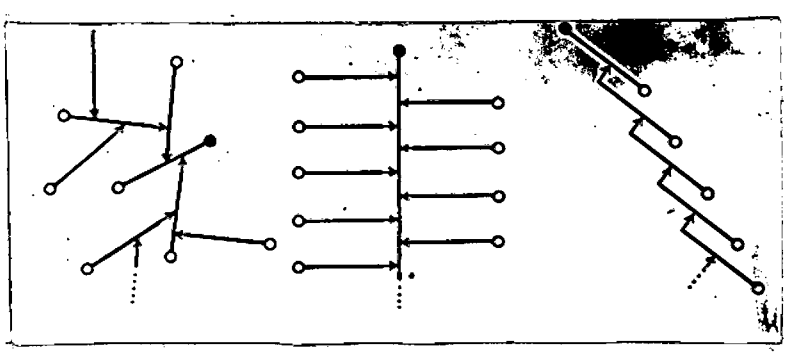
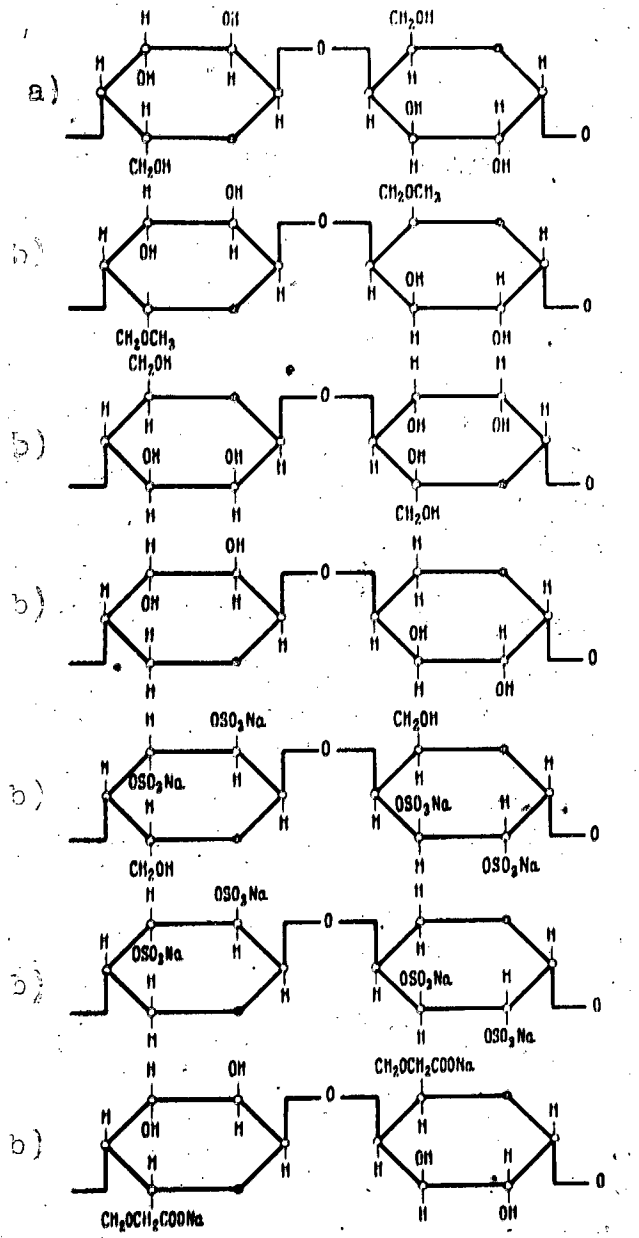


Fig.6 Molecular structures for glycogen.1)"Tree"form (Meyer);2)"Comb"form(Staudinger);3)"Laminated"form (Haworth).Linear chain represents 1:4 linked D-glucose residues. ⟶ represents interchain linkage type,1:6 in form nos.1 and 3.In form no.2 glucosidic linkage of type 1:2;1:3 and 1:6 is postulated. R represents free reducing group.(Greenwood,1956).



Cellulose

Methylcellulose

Mannan

Xylan

Cellulose-sulphuric acid ester

Xylan-sulphuric acid ester

Cellulose-glycolic acid ether

Fig.3

a) Basic unit and b) structure of some linear macromolecules used

Table 3

Polysaccharide derivatives used to study their chemical structure and biological activity

Substance	Physico-chemical properties
Methylcellulose	Surface active, homopolar, unbranched molecules.
Mannan and Xylan	No surface activity, homopolar, unbranched molecules.
Cellulose-glycolic acid ether	Weak heteropolar, unbranched molecules.
Xylan-sulphuric acid ester and Cellulose-sulphuric acid ester	Strong heteropolar, unbranched molecules.
Glycogen	Homopolar, strongly branched molecules.
Dextran	Homopolar, branched molecules.
Methylamylopectin	Homopolar, branched molecules.
Oxyethylamylose	Homopolar, linear molecules.
Oxyethyl Starch From Maize Starch which is composed of 25% Amylose and 75% Amylopectin; (Kerr, 1945; Kerr, Cleveland and Katzbeck, 1951).	Homopolar, branched molecules.

B. BRIEF SUMMARY OF SOME POLYSACCHARIDE DERIVATIVES: THEIR CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITY

It is possible to correlate the chemical structure of some macromolecules with their biological activity. As polysaccharides are much better understood than any other high polymer substances the first macromolecules to be investigated were xylan and cellulose (Husemann et al., 1947; Wiedersheim et al., 1953). The effect of these substances on the white blood cell count, on the erythrocyte sedimentation rate, on body temperature, on blood pressure, and on respiration, and their reabsorption in body tissues were studied. The substances were used in different degrees of polymerisation and are listed in Table 3. The structures of the substances listed in Table 3 are given in Figs. 3, 4, 5, and 6. The stereochemical formula of OES is given on page 22. Those of methyl and oxyamylopectin are the same as that of OES, but the hydrogen atom at the CH_2OH group is replaced by a methyl (CH_3) radical and an ethylene oxide molecule (CH_2-O-CH_2) respectively.

Action on the white blood cell count

The form of the molecule as well as its polarity influences the white blood cell count. All homopolar substances, whether of low or high degree of polymeri-

sation, cause leucopenia whereas strongly heteropolar substances evoke leucocytosis. Weak heteropolar compounds do not change the normal white cell count.

Action on the Erythrocyte Sedimentation Rate

The polarity and length of the molecules influence the sedimentation rate of red blood cells. Homopolar compounds of more than 1,500 Å in length increase the erythrocyte sedimentation rate whereas strong heteropolar substances inhibit it. Weak heteropolar compounds do not alter the erythrocyte sedimentation rate.

Action on the body temperature

The injection of unbranched and branched homopolar molecules raises the body temperature. Some heteropolar substances are pyrogenic in their higher degrees of polymerisation.

Action on blood pressure and respiration

Apart from cellulose-sulphuric acid ester of a high degree of polymerisation none of the substances detailed above change either the blood pressure or the respiration rate in animals. The reabsorption of substances having such chemical structures is dependent on the degree of polymerisation and substitution and not on the degree of ramification.

As more and more substances of macromolecular character are used in medicine, it becomes of interest

to search for compounds which have as few undesirable properties as possible. Cellulose derivatives, for example, are not metabolized by body enzymes, and repeated injections of proteins often cause allergic reactions. The ideal substance should be non-toxic, non-allergy-producing, easily obtained, and cheap. A water-soluble starch derivative, oxyethyl starch, showed encouraging results and the bulk of this thesis is devoted to a description of its production, pharmacological properties, and physiological effects.

Table 4 (Continued)

D) Other high polymer substances. (Fractions of starch and cellulose derivatives.)

Fraction	Substance	Time of degradation	Viscosity number $Z\eta$	Degree of polymerisation	Molecular weight
1	Amylose	not degraded	0.1980	1151	186,450
2	Oxyethyl-amylose	4 hours	0.0425	470	96,800
3	Amylopectin	not degraded	0.0728	720	130,300
4	Oxyethyl-amylopectin	not degraded	0.0934	935	209,400
5	Methyl-amylopectin	2 hours	0.0210	200	39,000
6	Methyl-cellulose	degraded commercially	0.500	500	85,000
7	Methyl-cellulose	3 hours	0.364	360	66,000
8	Methyl-cellulose	13 hours	0.165	165	30,000
9	Methyl-cellulose	48 hours	0.050	50	8,500
10	Xylan-sulphuric acid ester	not degraded taken from raw xylan DP 150	0.045	100	33,600
11	Xylan-sulphuric acid ester	taken from degraded xylan with a DP 50	0.004	12	5,300
12	Mannan	taken from bulbs of tubera salep	0.060	400	64,000
13	Cellulose-glycolic acid ether	taken from ramie fibres	0.072	600	144,000

Table 4

List of polysaccharide derivatives prepared

A)
Maize starch degraded hydrolytically at 38°C in N-methanol/HCl.

Fraction	Time of degradation	Viscosity number $Z\eta$	Degree of polymerisation	Molecular weight
1	8 min.	0.1704	2840	460,100
2	10 "			
3	15 "	0.0869	1440	233,280
4	18 "			
5	30 "			
6	1 hour			
7	2.5 hrs.			
8	3 hours	0.0466	840	136,100
9	5 "			
10	7 "			
11	9 "	0.0202	330	53,460
12	12 "	0.0126	210	34,020

B)
Oxyethylstarch, using raw starch and various fractions of hydrolytically degraded maize starch.

Fraction	Time of degradation	Viscosity number Z	Degree of polymerisation	Molecular weight
1	8 min.	0.2022	2000	412,000
2	2.5 hrs.	0.0922	920	188,500
3	3 hours	0.0508	500	103,000
4	5 "	0.0472	470	96,800
5	7 "	0.0305	300	61,800
6	9 "	0.0169	170	35,000
7	12 "	0.0096	100	20,000

C) OES labelled with ^{14}C

Fraction	Time of degradation	Viscosity number Z	Degree of polymerisation	Molecular weight
1	8 min.	0.0460	460	94,700
2	15 "	0.0869	870	179,200
3	2.5 hrs.	0.0902	900	185,400
4	7 hours	0.0190	190	59,140

C. PHYSICAL PROPERTIES OF SUBSTANCES INVESTIGATED AND A DESCRIPTION OF THE METHOD OF VISCOSIMETRY USED

A list of all the fractions of oxyethyl starch (OES) and of some other compounds is given in Table 4 and is followed by a short description of the meaning of the different symbols used to express the viscosity and chain-length of the molecules. A description of the preparation of OES is given on page 21 . The preparation of methylcellulose, mannan, xylan-sulphuric acid ester, cellulose-sulphuric acid ester, and cellulose-glycolic acid ether was carried out in conjunction with Husemann et al. (1954). The viscosity of all these solutions was measured with Ostwald viscosimeters of varying capillary width, and the degree of polymerisation (DP) was calculated according to Staudinger's equation:

$$DP = \frac{Z\eta}{Km}.$$

$Z\eta$ represents the viscosity number of a given solution (Schulz and Blaschke, 1941). ηrel expresses the relative viscosity which is the ratio of the speed developed by a given solution to that developed by its solvent passing through the capillary of the viscosimeter. In other words, ηrel equals the viscosity of the solution (t_1) multiplied by its density (d_1), divided by the viscosity of the

solvent (t_o), multiplied by its density (d_o).

Thus $$\eta \text{ rel} = \frac{t_1 \times d_1}{t_o \times d_o}$$

η sp is the specific viscosity i.e. the increase of the intrinsic viscosity which occurs when a given substance is dissolved in a given solvent.

Thus $$\eta \text{ sp} = \eta \text{ rel} - 1$$ (Staudinger and Heuer, 1930)

The concentration of the solution whose η sp is to be estimated is given as c. The specific viscosity (η sp) depends on and increases with the concentration (c) of the solution measured but η sp of linear molecules with a higher degree of polymerisation does not increase quite proportionally with the concentration (c).

A method of calculating η sp at infinite dilution can be applied, taking a series of viscosity measurements on solutions of the substance at different concentrations; the η sp values of the solutions are then extrapolated graphically.

Thus, the viscosity number $Z\eta = \lim_{c \to o} \eta \text{ sp}$ where c is expressed in g/L. In the American literature one frequently finds the unit η representing the intrinsic viscosity where c is expressed in g/ml., (Kraemer and

Table 5

List of Km values of substances used

Substance	Km value	Solvent	Author
Methylcellulose	10×10^{-4}	0.5 M NaCl	(Schulz, 1936)
Mannan	15×10^{-4}	" " "	(Husemann, 1940)
Xylan sulphuric acid ester	3×10^{-4}	" " "	(Pfannemueller, 1953)
Cellulose sulphuric acid ester	6.3×10^{-4}	" " "	(Husemann et al., 1946)
Cellulose glycolic acid ether	12×10^{-4}	" " "	(Husemann et al., 1954)
Amylopectin	1×10^{-4}	" " "	(Lansky, Kooi and Schoch, 1949)
Amylopection	0.85×10^{-4}	Chloroform	(Staudinger, 1950)
Oxyethyl-amylopectin	approx. 1×10^{-4}		
Amylose	0.9×10^{-4}	Formamide	(Pfannemueller, 1949)
Maize starch	0.63×10^{-4}	Formamide	(Staudinger and Husemann, 1937)
Oxyethyl-starch	approx. 1×10^{-4}		

Lansing, 1935). Thus: η sp = $Z\eta$ × 1,000.
$Z\eta$ values vary in proportion to the number of the chain-forming atoms.

The degree of viscosity of a colloidal solution was and still is given in poise or centipoise units. These represent absolute values and their use ought to be reserved to express the viscosity of pure liquids only, as absolute viscosities of colloidal solutions vary with concentration. More recently, the relative units of η rel and η sp and the derived $Z\eta$ have been used to estimate the viscosity of colloidal solutions and, in the case of linear molecules, their degree of polymerisation.

As the equation $DP = \frac{Z\eta}{Km}$ indicates, each viscosity number is divided by a constant Km which varies according to the substance measured. Table 5 gives the Km values, which are usually calculated by estimating the DP osmotically and viscosimetrically. The Km of OES and oxyethylamylopectin are not estimated but one can safely assume that it will not differ much from amylopectin as the heterogenous starch contains about 80% amylopectin molecules.

The method of using a viscosimeter is simple. The temperature of the solution measured must be constant and this is best achieved when the viscosimeter is put

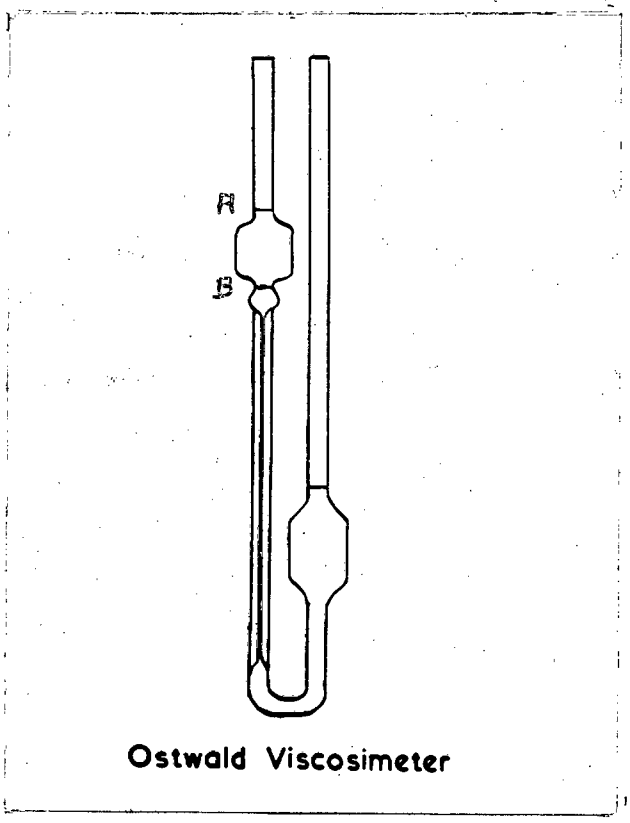


Fig.7

Standard viscosimeter for measuring the degree of polymerisation of linear polysaccharides

into a water-bath with thermostatically regulated temperature. The viscosimeter, especially its capillary, must be kept strictly clean. This applies also to the solution measured. The viscosimeter must be fixed in a vertical position when put into the water-bath and enough fluid should be placed into it to ensure that the upper vessel can be filled without emptying the U-tube below the lower vessel when the solution is pumped from the latter to the former. The capillary of the viscosimeter used to measure the various substances ought to be of such width, or the solutions to be measured of such concentration, that t_1, which is the time in which the solution passes from A to B, does not exceed 5 minutes and t_o, which is the time in which the solvent passes from A to B, is not more than 150 seconds and not less than 70 seconds. Fig. 7 shows an Ostwald viscosimeter used to estimate the viscosity of linear molecules or molecules which show an increased viscosity with an increase in length of their molecules. Their molecular weight may be estimated according to the formula $DP = \frac{Z\eta}{Km}$ whereby $Z\eta$ can be calculated using the equation $\lim_{c \to o} \eta sp = Z\eta$ or by applying the formula of Schulz and Blaschke (1941), $Z\eta = \frac{\eta sp/c}{1 + (K\eta \times \eta sp)}$ which makes an extrapolation superfluous. Colloidal

solutions, whose viscosities are independent of their degree of polymerisation and their structure, are not linear but usually spherical in shape, as for instance glycogen or many proteins. The viscosity of such solutions cannot be measured in an Ostwald viscosimeter, and their DP cannot be calculated according to Staudinger's formula ($DP = \frac{Z\eta}{Km}$) since ηsp does not change with a change of the DP. Solutions of such nature usually fulfil Einstein's equation $\frac{\eta sp}{c} \times d = 0.0025$ where d is the density of the solution (Einstein, 1906, 1911; Staudinger and Husemann, 1935). Polson (1956) however, working on the determination of molecular weights of various proteins, found that $\frac{\eta sp}{c} \times d$ did not equal 0.0025 but that this figure has to be raised to 3.27 when the relationship is applied to dissolved proteins. The estimation of the degree of polymerisation or of the molecular weight of substances which obey the Einstein equation can be carried out in various ways: osmotically, with the ultracentrifuge, using the light scattering method, or by means of a special viscosimeter i.e. Ubbelhode's type or a modification of it (Davis and Elliot, 1949).

D. OXYETHYLSTARCH AND ITS COMPARISON WITH OTHER CELLULOSE DERIVATIVES

PREPARATION OF WATER-SOLUBLE STARCH

Degradation of starch

Maize starch was used as a raw material. 100g. of maize starch was degraded hydrolytically with 100ml. of N-HCl in methanol at 38°C. The fraction of degraded starch obtained depended on the duration of hydrolysis. After the starch had been neutralized by washing in distilled water it was dried at 38°C. The degree of polymerisation was measured with an Ostwald viscosimeter and calculated according to Staudinger's equation $DP = \frac{Z\eta}{Km}$, where DP is the degree of polymerisation, $Z\eta$ is the viscosity number of the substance measured, and Km is a constant. The Km for starch, measured in formamide, is 0.63×10^{-4} (Staudinger and Husemann, 1937). The longer the starch is degraded in the N-HCl/methanol, the smaller becomes $Z\eta$ and hence also the DP and the molecular weight.

In the present investigation 5 fractions of starch, degraded for 8 min, 2.5, 5, 7, and 9 hours were used. None of these products are water-soluble; they are starch molecules with different degrees of polymerisation, i.e.

with different molecular weights. Their degrees of polymerisation, when measured viscosimetrically in formamide as a solvent, were in the range of 2,800 for the highest polymer fraction (8 min degraded) and 330 for the lowest (9 hr degraded); their molecular weights were 460,000 and 53,460 respectively (Table 4 above).

Production of oxyethylstarch

According to Ziese (1935), water-soluble derivatives of starch can be obtained by treating the starch with ethylene oxide. In our laboratory 40g. of starch were made into a paste with 25ml. of water and then mixed with 800ml. of N-NaOH and kept for 4 hours, in an atmosphere of nitrogen. This is of importance as it is known (Baum and Gilbert, 1954) that amylose contains oxygen-sensitive bonds and is rapidly broken down to smaller units (Whistler and BeMiller, 1958). This cannot be said of any starch/NaOH solution when kept at 0°C or in an atmosphere of nitrogen (Schoch, Wilson, and Hudson, 1942; Whistler and Johnson, 1948; Witnauer, Senti, and Stern, 1952). Nitrogen was bubbled through the solution for 15 min, which prevents the starch from degrading further as practically all the oxygen is expelled. 20g. of ethylene oxide (boiling point 10.4°C) was then introduced in a

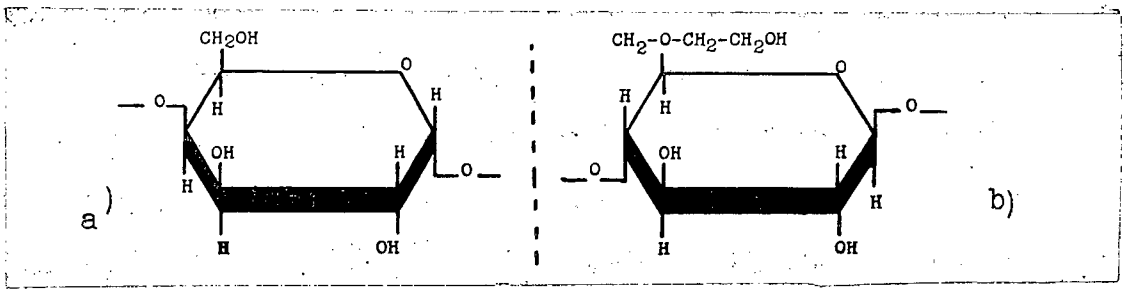


Fig.8

Unit of a starch and of an oxyethylstarch molecule
a) starch b) oxyethylstarch

gaseous form into the starch/NaOH solution for 30 min, in the first experiments at room temperature, and later at +4 to +8°C. The product was neutralized with 2N-HCl and dialyzed with distilled water until no free Cl^- ions were detectable with $AgNO_3$. The Cl-free solution was concentrated under a stream of warm air, precipitated with acetone, dried at 38°C, and powdered. The product obtained was oxyethylstarch (OES). The structure of a glucose unit before and after the addition of ethylene oxide is given in Fig. 8. The degree of substitution of OES can be estimated, using the starch iodine reaction i.e. blue, in the case of unsubstituted starch, with changes through violet and reddish-brown to yellow as the degree of substitution of the basic unit with ethylene oxide increases. The degree of polymerisation was measured again, this time in 0.5 M-NaCl. The values were 2,000, 920, 470, 300, and 170, representing a molecular weight of 412,000, 188,500, 96,800, 61,800, and 35,000 respectively. Itmay be argued that the values viscosimetrically obtained are not sufficiently accurate to work out a correct degree of polymerisation or to estimate the molecular weight of a given substance. Therefore comparative values of degrees of polymerisation obtained with the ultracentrifuge, the viscosimeter, or

Table 6

Comparison of values of the degrees of polymerisation of methylcellulose obtained using viscosimetry and ultracentrifuge

CH_3O % in methylcellulose	Degree of polymerisation	
	Ultracentrifuge	Viscosimeter
22.6	80	70
22.8	130	135
31.7	200	200

Table 7

Comparative values obtained to estimate the length of cellulose nitrate molecules using different methods.

L is the length of the molecule in Å units and M the molecular weight.

L roentgenographic	L viscosimetric	L ultracentrifuge	M/osmotic
129	152	140	6,200
582	756	538	30,000
1535	1486	1380	80,000
3868	3066	2846	119,000

by roentgenographic methods are given in Tables 6 and 7 (Staudinger, 1950). From these figures it may be seen that the viscosimetric method used to calculate the degree of polymerisation of linear molecules is reasonably accurate. Ubbelhode's viscosimeter is suitable for measuring the molecular weight of spherical molecules, while Ostwald's viscosimeter is used for measuring the linear type. As starch, and thus OES, is a mixture of linear amylose and branched amylopectin (Meyer and Bernfeld, 1940; Meyer and Gibbons, 1950) it was necessary to verify that ordinary viscosimetry could be applied using Ostwald's viscosimeter instead of Ubbelhode's.

The viscosity of starch alters with a change in the size of its molecules, otherwise the equation $DP = \frac{2\eta}{Km}$ would not be applicable and Ostwald's viscosimeter would be of no use. On the other hand, the viscosity of glycogen, which is a strongly branched or spherical molecule (Manners, 1957; Meyer, 1943), does not alter when its DP is measured viscosimetrically using Ostwald's viscosimeter. Solutions of these molecules, of the same concentration but of a different DP, possess the same viscosity and it is impossible to apply Staudinger's equation in these cases. Einstein's equation

Table 8

Comparative values of the specific viscosity (ηsp) of cellulose, starch and glycogen in their varying degrees of polymerisation

Degree of polymerisation (DP)	ηsp of: Cellulose Km 5×10^{-4}	ηsp of: Starch Km 0.63×10^{-4}	ηsp of: Glycogen
400	32.4	4.1	1.4
1700	137.0	17.3	1.4
5000	405	51.0	1.4

($\frac{\eta sp}{c} \times d = 0.0025$) should be applied to prove that these molecules are spherical in shape and that no constant ratio between $Z\eta$ and DP can be determined.

Unlike glycogen, cellulose and its derivatives possess a strict linear shape and do not fulfil Einstein's equation, but their DP can be estimated by applying the Staudinger formula ($DP = \frac{Z\eta}{Km}$) as the viscosity of solutions of these molecules does change with a change in their size.

It appears then that starch, as far as its molecular shape and its viscosimetric properties are concerned, ranges between the linear cellulose and the spherical glycogen. Comparative estimations of the specific viscosity (η sp) of cellulose, starch, and glycogen show that cellulose and starch do not give a constant viscosity number when measured in various degrees of polymerisation. On the other hand, viscosity measurements of glycogen of various degrees of polymerisation always show the same values. As there is a clear increase in $Z\eta$ when the degree of polymerisation increases, the equation $DP = \frac{Z\eta}{Km}$ can be applied for starch and cellulose but not for glycogen. Table 8 shows comparative values between the η sp of cellulose, starch, and glycogen which have the same DP, (Staudinger and Husemann, 1937).

Production of radioactive OES

The storage and elimination of OES was investigated using OES labelled with ^{14}C. According to the Radiochemical Centre in Amersham, England, the ^{14}C used was isolated as starch ^{14}C from tobacco leaves. The starch was produced by photosynthesis in the presence of ^{14}C-CO_2 for 10 - 12 hours. After the leaves had been extracted with 80% ethanol, they were macerated in warm water and ground up to release the starch. The solution was diluted with inactive water-soluble starch and the polysaccharide was precipitated as its iodine complex. The iodine was removed from the iodine complex with alkali before the starch was finally dried. The specific activity was given as 62 µc/mg. Altogether 6mg. of starch-^{14}C was mixed with 30g. of degraded starch (degradation time, 8 min) by dissolving the latter in the usual way and using N-NaOH as the solvent. The powdered ^{14}C preparation from tobacco leaves was then added and the ^{14}C distributed, the solution being carefully and thoroughly mixed with the help of nitrogen gas bubbling through the mixture.

As it was not quite certain whether the small quantity of 6mg. of ^{14}C would be evenly distributed in 30g. of non-radioactive starch, samples of the starch were taken

Table 9

Radioactivity of OES-^{14}C(DP 460) when measured as starch/NaOH solution in dialyzing tubes

Sample number		Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m/g.
Surface	1	0.2635	5	7171	1434	1435	5446
	2	0.2188	5	6159	1232	1230	5622
Middle	3	0.1864	1	1236	1236	1234	6620
	4	0.2883	1	1936	1936	1945	6746
Bottom	5	0.2488	1	1593	1593	1597	6419
	6	0.2734	1	1770	1770	1777	6500

G.M.counter, EHM, No. 5154.EHT 1320 volt
Perspex plate and tray in position No. 1
End window, lead-castle.

The background count was

$\frac{265}{30}$ = 9 c.p.m.

Table 10

Radioactivity of OES-^{14}C(DP 460) when measured as the final powdered product

Sample number	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m/g.
1	0.2648	2	2027	1014	1010	3814
2	0.2488	2	1987	994	990	3979
3	0.2734	2	2212	1106	1103	4034
4	0.2759	2	2177	1089	1086	3936

The background count was 9 c.p.m.

at different stages of the production of OES-^{14}C. The β -emission of the first samples was measured after the starch/NaOH solution was believed to be adequately mixed and after it had been neutralized with 2N-HCl (Table 9). The samples were taken while the starch/NaOH solution was still in the dialyzing tube. Samples 1 and 2 were taken from the surface, samples 3 and 4 from the middle, and samples 5 and 6 from the bottom layer of the solution. When the OES had been concentrated, precipitated, dried, and powdered as described on page 22, it was tested again for its β -emission. This time the samples were taken at random from the container, ground again into a very fine powder, and the sampling-trays filled. The counting device and method were the same throughout the experiments summarised in Tables 10 - 15. The background counts were carried out over a period of 20 minutes or longer. The weights are given in grams. Table 10 gives the β -emission of the OES powder. The dried OES showed less activity per gram than the neutralized starch/ethylene oxide solution. It had to be determined whether any ^{14}C had escaped from the dialyzing tube while the starch was being dissolved in NaOH and neutralized with HCl, or whether this decrease in radioactivity was due to the

Table 11

Radioactivity of the water in which the dialyzing tubes were immersed and of the acetone used to precipitate the dissolved OES

Sample number	weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m/g.
1) H_2O	0.2412	3	79	26	17	70
2) H_2O	0.2634	3	73	24	15	57
3) H_2O	0.2691	1	10	10	-	-
4) H_2O	0.2629	5	45	9	-	-
5) Acetone	0.0580	15	360	24	15	
6) Acetone	0.0402	5	188	37	28	

The background count was 9 c.p.m.

precipitation of the OES in acetone. Samples of water, in which the dialyzing tubes were immersed, and of acetone used to precipitate the OES, were taken and the beta-emission counted in the same way as mentioned above. Samples 1 and 2 were taken 6 hours after the dialyzing tubes had been put into the water, samples 3 and 4 were taken 14 hours later. Samples 5 and 6 were the acetone washings used for precipitating the OES.(Table 11)

The radioactive OES of a degree of polymerisation of 460 was dissolved in 0.9% saline and injected into rabbits weighing 2.8 - 3.0kg. The amount injected was 10ml./kg., the concentration of the OES, 3%. Seven hours after the injection a rabbit was killed with Nembutal and the organs to be examined removed, minced, washed in acetone, dried over-night at 55°C, and then powdered. Blood taken from the vena cava, urine from the bladder, and faeces from the colon, were also examined. The blood cells, dried serum, and faeces were powdered and the concentrated urine was examined as a thick brown smear. Aliquots of the pulverized organs were transferred into special trays and their beta-emission recorded. As the pulverized organs showed either a coarser or a finer surface after being ground up, and sometimes an uneven surface when put onto the tray, it was not surprising that the values obtained varied and

Table 12

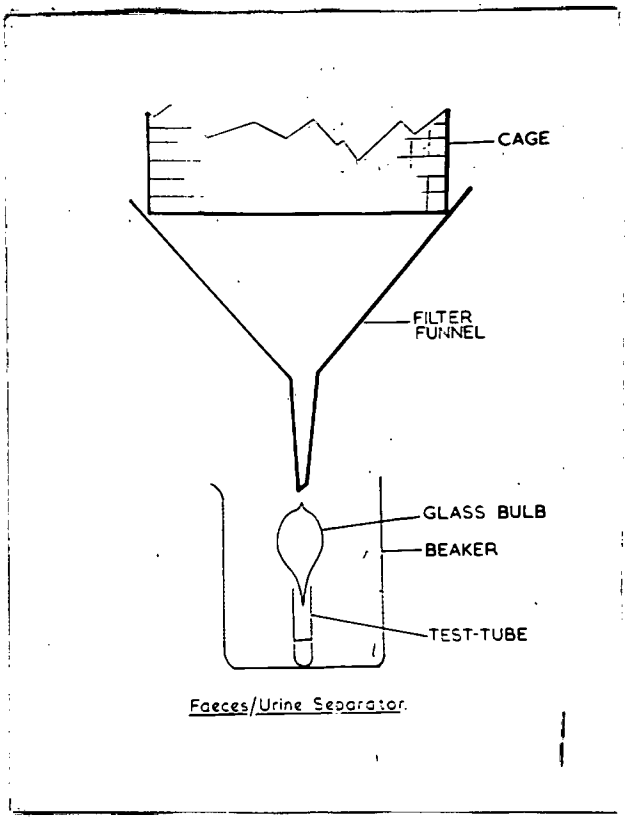
Radioactivity in various organs of a rabbit (2.8 kg) injected with 10 ml. 3% OES-^{14}C(DP 460) solution

Sample number		Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1.	Clotted	0.4863	3	27	9	-	-
2.	blood	0.6620	2	21	11	-	-
3.	Plasma	0.5704	2	18	9	-	-
4.	"	0.6895	3	33	11	-	-
5.	Faeces	0.4878	2	26	13	-	-
6.	"	0.5731	2	22	11	-	-
7.	Urine	1.0638	5	3337	667	658	619
8.	"						
9.	Lung	0.3941	2	20	10	-	-
10.	"	0.3122	2	18	9	-	-
11.	Kidney	0.4236	4	50	13	-	-
12.	"	0.3914	4	50	13	-	-
13.	Muscle	0.4410	2	18	9	-	-
14.	"	0.3712	2	16	8	-	-
15.	Liver	0.5886	2	19	10	-	-
16.	"	0.4826	2	22	11	-	-
17.	Spleen	0.1238	2	21	11	-	-
18.	"						
19.	Acetone	-	2	-	-	-	-
20.	"	-				-	-
21.	Filter-	-	2	24	12	-	-
22.	paper	-	-	-	-	-	-
23.	OES	0.5200	2	13134	6567	6921	13320
24.	"	0.4130	2	13013	6507	6863	16593

Background: 9 c.p.m.

were not absolute. Counts of beta-emission of samples slightly nearer to the end-window were higher than for those previously measured and great care was taken to have as homogeneous a spread and as evenly ground tissue samples as possible. The organs were washed in acetone and, to find out whether any loss in activity might be caused by this process, the filter paper and acetone were kept and their possible beta-emission measured. Finally samples of OES-^{14}C of DP 460 were put onto a tray and counted. The results of these end-window counts are given in Table 12.

It is obvious that a weak beta-ray activity cannot be measured accurately with an end-window counter as the self-absorption of beta-emitters is rather high. A small increase of the counts above the background counts would indicate some radioactivity of the organs under investigation. The fact that only the urine, and none of the organs investigated, showed any OES-^{14}C did not necessarily mean that there was no radioactivity present in the organs. The experiment was therefore repeated, but a 4% instead of a 3% solution of OES-^{14}C was used and 30ml. instead of 10ml. injected. Seven hours later the rabbit was killed with Nembutal, the organs processed as described above, and their radioactivity measured. Samples of urine and faeces were obtained by placing the



Faeces/Urine Separator.

Fig.9

Table 13

Radioactivity in various organs of a rabbit (3.0 kg) injected with 30 ml. 4% OES-^{14}C(DP 460) solution

Sample number	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1. Liver	0.3326	3	66	22	11	33
2. "	0.2819	3	67	22	11	39
3. Kidney	0.2465	2	143	72	61	247
4. "	0.2723	2	132	61	50	184
5. Heart	0.2072	4	51	13	-	-
6. "	0.2449	3	39	13	-	-
7. Blood	0.3680	2	17	9	-	-
8. "	0.2963	2	27	14	-	-
9. Plasma	0.3865	3	52	17	6	16
10. "	0.2479	3	54	18	7	28
11. Muscle	0.4435	2	24	12	-	-
12. "	0.2957	2	21	11	-	-
13. Lung	0.2543	2	16	8	-	-
14. "	0.3187	2	24	12	-	-
15. Spleen	0.2576	11	210	19	8	31
16. Urine	0.9329	2	3202	1601	1603	1718
17. "	0.5852	2	719	360	350	598
18. "	0.0980	2	113	57	46	469
19. "	0.1620	3	33	11	-	-
20. Faeces	0.4322	3	43	14	-	-
21. "	0.4415	3	29	10	-	-

Background: 11 c.p.m.

Urine samples nos. 16, 17, 18 and 19 were taken after 1, 2, 4 and 7 hours respectively.

rabbit in a metabolic cage and using a faeces-urine separator device. Such a device consists of a funnel placed under the animal's cage. The tip of the funnel is above the top of a glass bulb the lower part of which is drawn into a narrow tail-end resting in a test tube. The glass bulb and test tube are placed in a beaker. The faeces fall through the funnel and bounce off the top of the glass bulb, thus missing the test tube, but are collected in the beaker. The urine flows from the funnel onto the surface of the glass bulb, runs down its sides, and finally drips from the lower tip of the tail-end of the bulb into the test tube. The results of this experiment are given in Table 13. As was expected, the liver and kidney showed some activity. The fact that the lung tissue did not show any radioactivity gave some indication that OES-^{14}C is not stored in the lung 5 - 10 hours after injection in sufficient quantities to be measured by the end-window method. A drawing of the urine-faeces separator is given in Fig. 9.

A more sensitive counting device, gas-vacuum, or scintillation method may show some activity in the lung; but since the end-window method gives good comparative figures, it is clear that OES is not stored primarily in the lung assuming that the absorption-curve for lung tissue is not very different from that of the liver.

Table 15

Radioactivity of OES-^{14}C (DP 190, 870 and 900) when measured as the final powdered product.

Sample number	DP of OES-^{14}C	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	190	0.014	1	4,246	4,246	4,394	313,857
2	190	0.012	2	8,965	4,482	4,663	388,583
3	190	0.042	1	6,443	6,443	6,804	162,000
4	190	0.042	1	7,056	7,056	7,478	178,047
5	900	0.022	1	9,255	9,255	10,010	455,000
6	900	0.028	1	9,130	9,130	9,893	353,321
7	900	0.020	1	8,347	8,347	8,962	448,100
8	900	0.016	1	8,039	8,039	8,617	538,562
9	870	0.036	2	10,940	5,470	5,697	185,250
10	870	0.025	2	10,212	5,106	5,314	212,560
11	Acetone	0.058	15	360	24	12	206
12	washings	0.040	5	188	37	25	625

Background: 12 c.p.m.

Table 14

Radioactivity of OES-^{14}C(DP's 190, 870 and 900) when measured as starch/NaOH solution in the dialyzing tubes

Sample number	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	0.0324	2	22,424	11,212	11,862	366,111
2	0.0444	2	23,458	11,729	12,449	280,383
3	0.0453	2	26,036	13,018	13,908	307,020
4	0.0734	2	10,723	5,362	5,502	74,959
5	0.0538	2	14,348	7,174	7,434	138,178
6	0.0898	2	14,285	7,143	7,393	82,327
7	0.0767	2	15,365	7,682	8,217	108,100
8	0.0902	2	11,978	5,989	6,305	70,071
9	0.0728	2	12,354	6,177	6,527	93,242

Nos. 1, 2 and 3 are the top, middle and bottom layers respectively of NaOH/OES-^{14}C solution degraded for 7 hours;

Nos. 4, 5 and 6 the same layers but for a NaOH/OES-^{14}C solution degraded for 2.5 hours; and

Nos. 7, 8 and 9 the same layers but for a NaOH/OES-^{14}C solution degraded for 15 min.

Background: 11 c.p.m.

The fact is that 7 hours after injection of OES-^{14}C no radioactivity of lung tissue was observed. This is in contradiction to the histological findings described later.

In order to obtain fractions of OES-^{14}C, the ^{14}C was linked with OES of various molecular weights. Altogether four samples were produced and degraded for 8 min.*, 15 min., 2.5hr., and 7 hr., with degrees of polymerisation of 460*, 870, 900, and 190 respectively. Samples were taken after ethylene oxide had been linked onto the starch molecule dissolved in N-NaOH and the solution neutralized with 2N-HCl and transferred into polythene dialyzing tubes. The results are given in Tables 14 and 15.

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*The surprisingly low degree of polymerisation of the OES-^{14}C prepared from starch degraded for only 8 min. is explained on pages 53 and 54.

TOXICITY

Acute toxicity after intravenous injection

The toxicity of high-polymer cellulose derivatives is difficult to prove: the rapid injection of highly viscous fluid may cause immediate death due to lung oedema, and prolonged infusion of high-polymer solutions may increase the intravascular volume, with resultant heart failure and death. The linear cellulose derivatives, methylcellulose, mannan, and xylan show no acute toxicity when injected in doses up to 100mg/kg. It is not possible to inject these substances in high concentration because the increased viscosity makes intravenous injection very difficult, and short term experiments were limited by this factor.

Branched strongly heteropolar substances such as xylan and cellulose-sulphuric acid esters were investigated by Husemann et al. (1947) and Wiedersheim et al. (1953). It was found that xylan-sulphuric acid esters, used as a synthetic heparinoid, caused internal haemorrhages when given in doses of 50mg/kg., that is, in 10 times its therapeutic dose. According to Husemann, Lötterle, Wiedersheim, and Hertlein (1954) cellulose-sulphuric acid esters showed a much greater toxicity

and the critical dose was 3mg/kg. It was observed that rabbits which survived 2.5mg/kg. could tolerate a much higher dose and some animals survived doses of up to 60mg/kg. (Wiedersheim et al., 1953). Weak heteropolar cellulose-glycolic acid ether did not show any toxicity and neither did glycogen and dextran which was tolerated by rabbits and rats when injected intravenously in doses of 10g/kg. Oxyethylstarch injected into dogs, rabbits, and rats was also found to be non-toxic in doses of 4g/kg.

Effect on blood pressure and respiration

The macromolecules mentioned above had very little effect on blood pressure and respiration: 10mg/kg. of homopolar linear high polymers did not change the blood pressure of dogs, cats, or rabbits when injected intravenously. On the other hand, strong heteropolar substances with a high degree of polymerisation did affect the blood pressure. This did not occur when the same substances with a low degree of polymerisation were injected. Cellulose-sulphuric acid esters of a DP of 500 caused a decrease of the arterial, and a slight increase of the venous blood pressure and the respiration became shallow. Repeated doses of increasing strengths

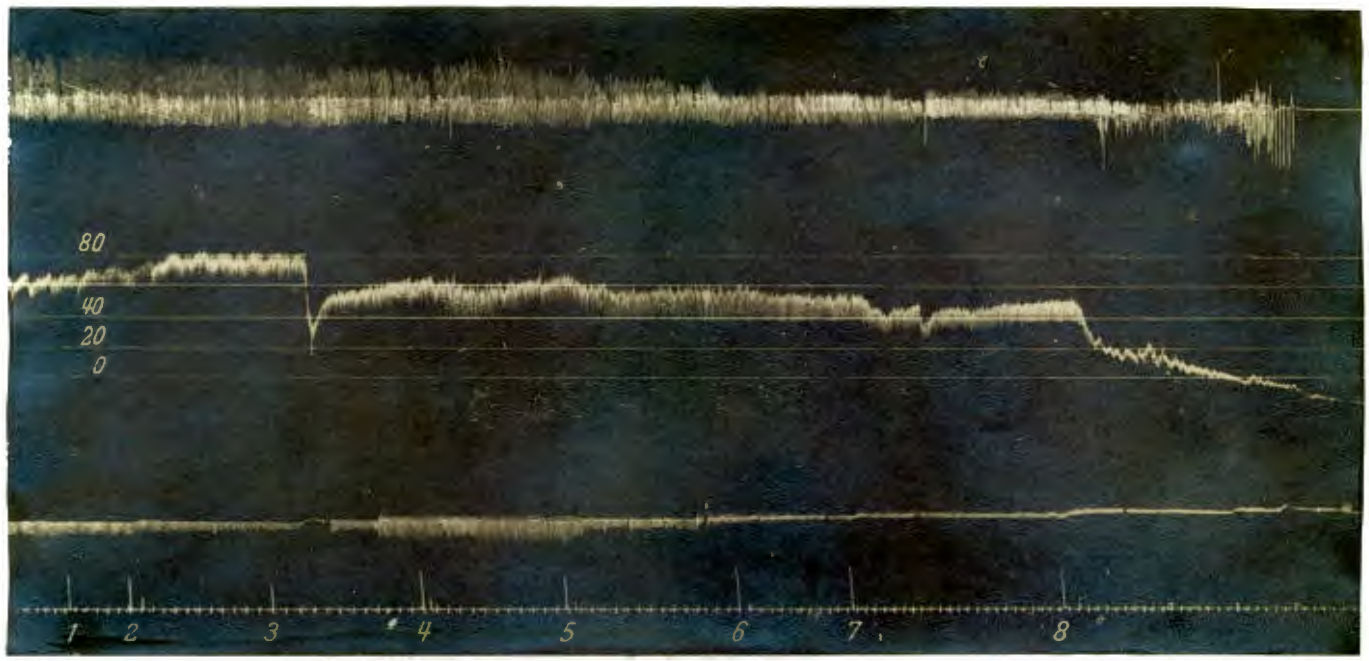


Fig.10 Effect of injection of cellulose-sulphuric acid ester on respiration and blood pressure.
Rabbit,2.7 kg.;paraldehyde anaesthesia.
Upper tracing: Respiration; Middle tracing:Arterial Pressure in mm.Hg.; Lower tracing: Venous Pressure; Time in 30 sec.
Nos.1-8 represent: 1. Injection of 2.0 ml 0.9% NaCl,
2-8.Injections ofcellulose-sulphuric acid ester,DP 500 .
2. 1.0 mg/kg.
3. 2.5 mg/kg.
4. 5.0 mg/kg.
5.10.0 mg/kg.
6. 20 mg/kg.
7. 40.mg/kg.
8. 80 mg/kg.

of cellulose-sulphuric acid esters did not cause any further alteration of the blood pressure even up to doses of 60mg./kg. and higher doses regularly caused the death of the animals. Fig. 10 is a record of one such experiment. Animals which were given xylan-sulphuric acid esters before injection of cellulose-sulphuric acid esters did not die when injected with amounts up to 80mg./kg. of the latter. These animals even tolerated 120-160mg./kg. Weak heteropolar cellulose-glycolic acid ether showed no toxicity. The branched molecules of glycogen, oxyethylstarch, methyl- and oxyamylopectin, dextran, and polyvinylpyrrolidone had no effect on blood pressure and respiration when injected slowly.

Absorption and high-polymer substances

As the substances tested varied in their polarity, surface tension, and degree of polymerisation and substitution, it was expected that differences in their rate and degree of absorption would be found.

All experiments were carried out on rats of 100-120g. in weight. These animals were anaesthetized with ether and into the hind-paw of each animal 0.5ml. of the solution under investigation was injected subcutaneously in a concentration of 1%. Five animals were used to test each substance. One animal received 0.9% NaCl

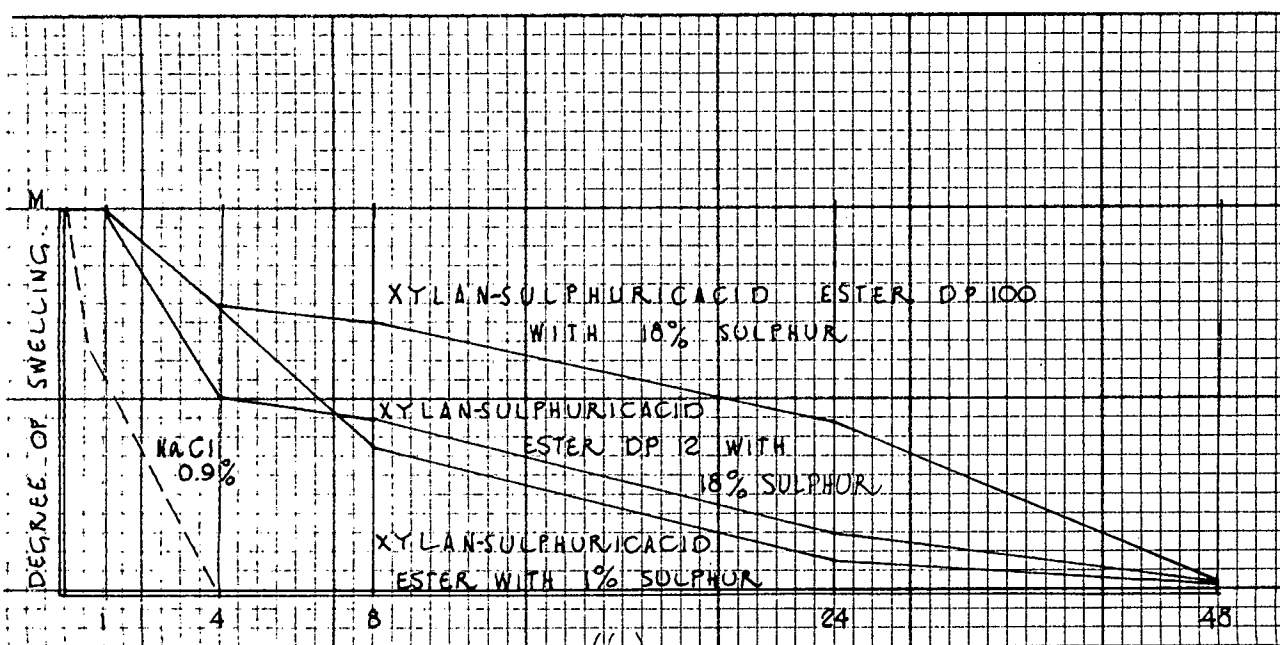


Fig.13 Absorption of xylan-sulphuric acid esters of various degrees of SO_3 substitution injected subcutaneously into rat paws

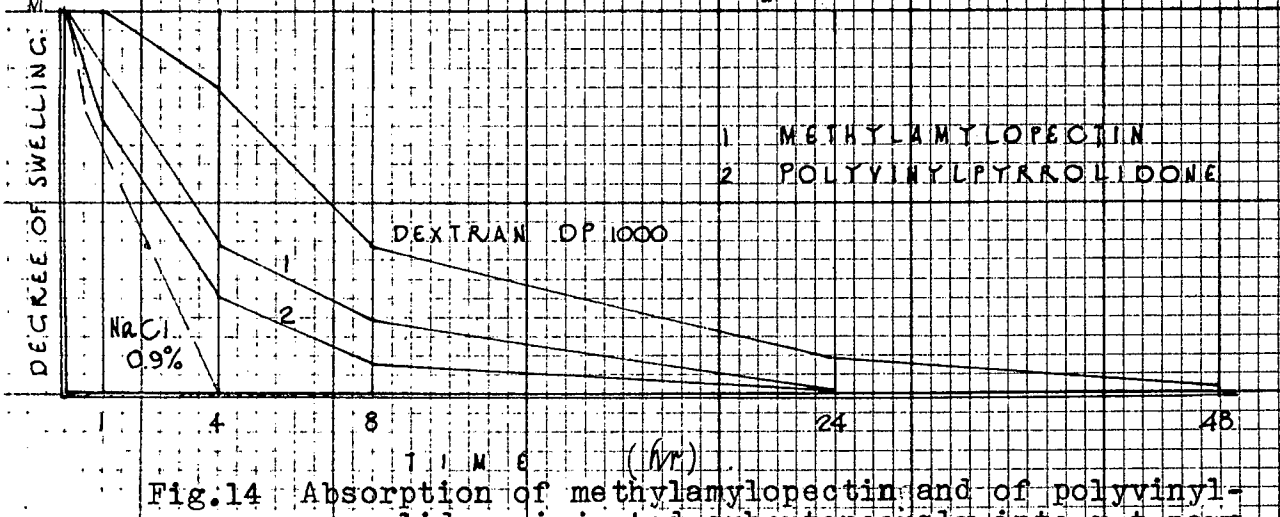


Fig.14 Absorption of methylamylopectin and of polyvinyl-pyrrolidone injected subcutaneously into rat paws

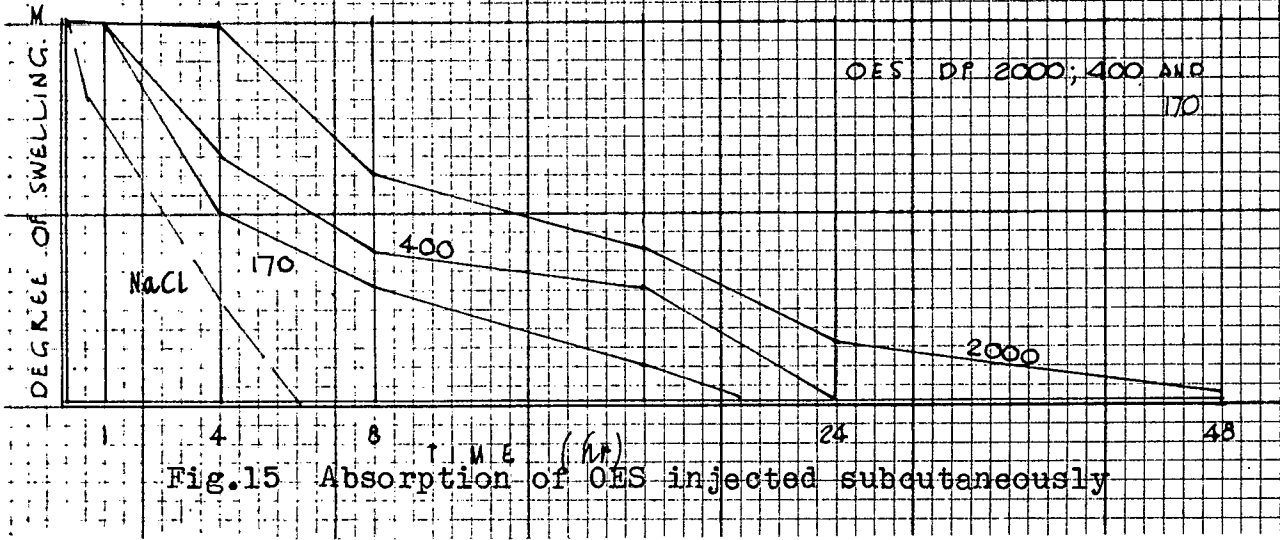


Fig.15 Absorption of OES injected subcutaneously

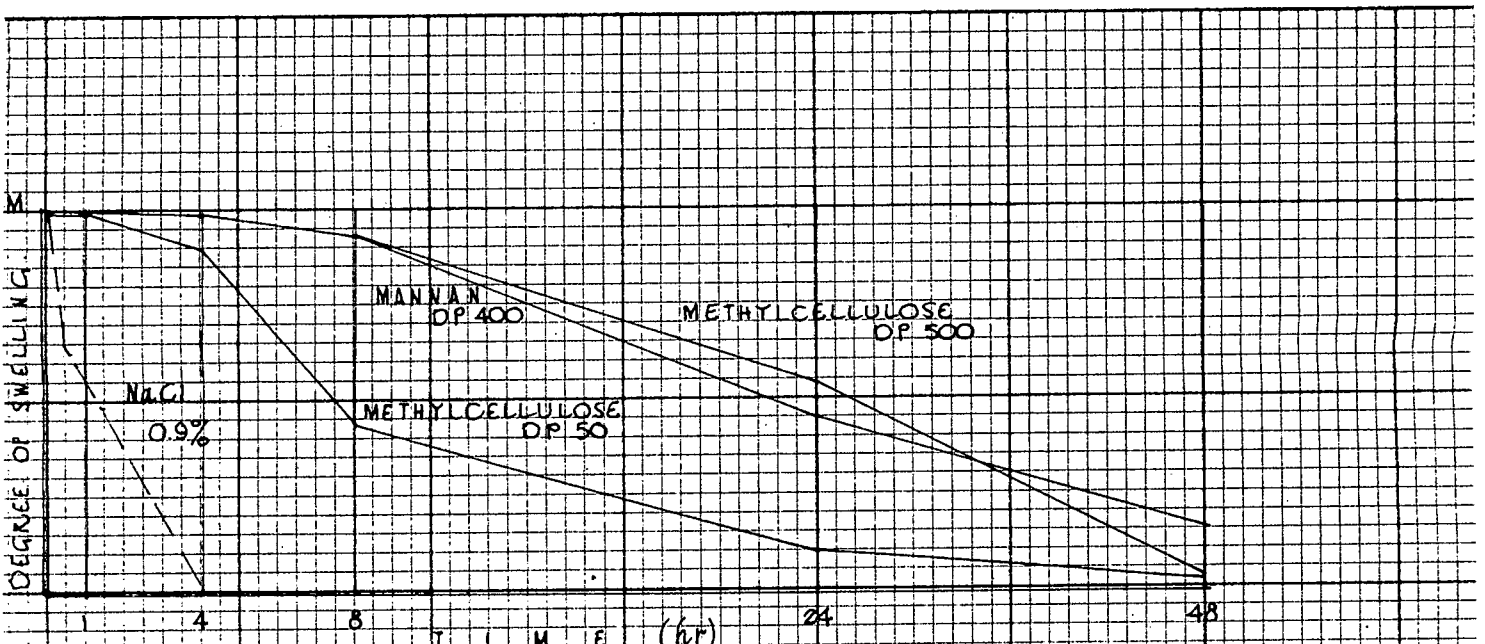


Fig.11 Absorption of mannan and of methylcellulose injected subcutaneously into rat paws

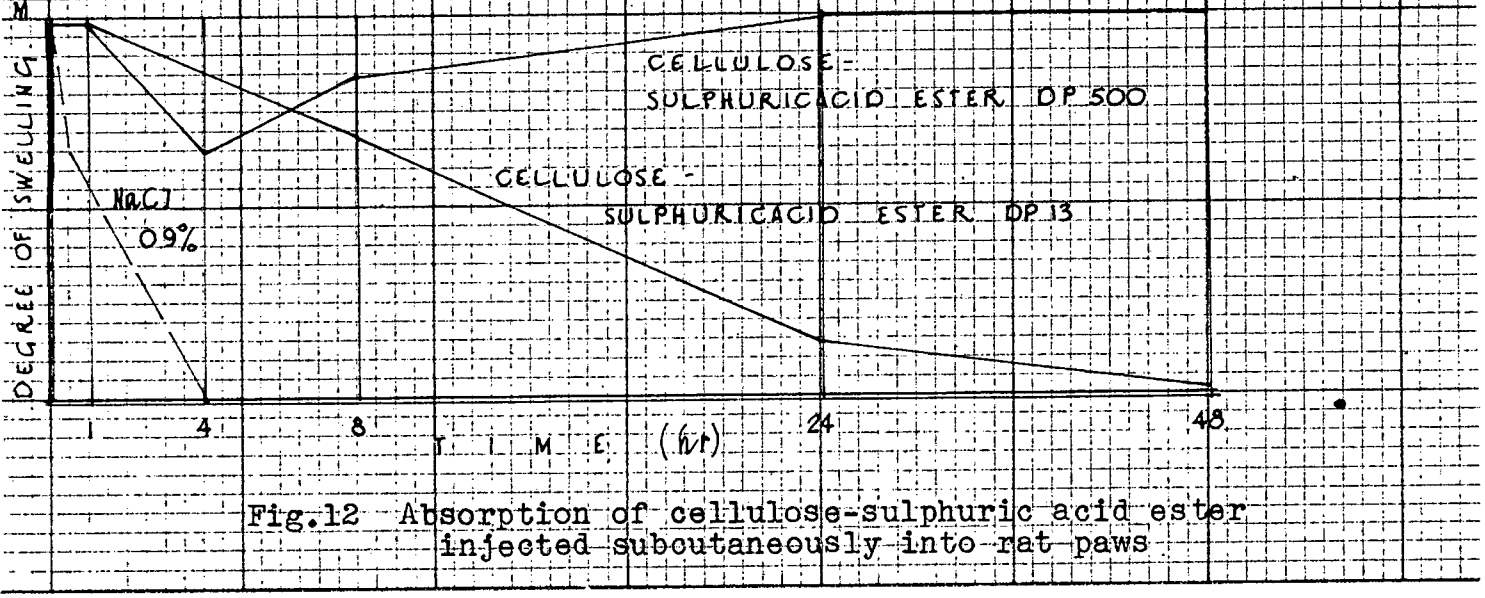


Fig.12 Absorption of cellulose-sulphuric acid ester injected subcutaneously into rat paws

(0.5ml.) and the untreated hind-paw was taken as the control. The degree of swelling of the paw was measured 1, 4, 8, 24, and 48 hours after injection and showed the rate of absorption of the substance. Maximum swelling of the hind-paw, as observed immediately after injection, is indicated in Fig. 11 at point M. Intermediate degrees of swelling were estimated by the degree of wrinkling of the skin on the injected paw and by comparing it with the control paw. The wrinkling of the skin increased with the increasing absorption of the solution injected. Figs. 11-15 show the rate of absorption of the various substances tested and the curves of 0.9% NaCl absorption used as a control.

Renal function and injection of OES

It was of interest to investigate whether samples of OES of various molecular weights would influence the activity of the glomeruli. Two experiments were set up to test the renal function in dogs and an infusion of OES (DP 2,000 and 300) was given; in both experiments the animals received 200ml. of OES as a 2% and a 4% solution respectively. The animals were anaesthetized with Nembutal (30mg./kg.) and creatinine clearance was carried out with sustained infusion of 0.1% creatinine solution in 0.85% saline at a rate of 5ml. per minute. Samples of blood and urine were taken at 30 min. intervals and the creatinine clearance calculated with a Dubosq

Table 18

Effect of OES on monkey kidney cells in tissue culture.

OES DP 2000		OES DP 170	
Conc. of OES in medium	Results	Conc. of OES in medium	Results
2%	Marked cytotoxic effect. Cells detached from glass and pyknotic. pH, 7.4.	4%	Cell sheets vacuolated. Cells markedly granular cytoplasm, but still adherent to glass. pH, 6.1.
1%	Some effect on tissue cells as above, but bulk of cells normal. pH, 6.6.	2%	Cells granular but sheet intact and attached to the glass. pH, 6.7.
0.5%	Occasional rounded cells but bulk of cells normal. pH, 6.8.	1%	Cells of normal appearance. pH, 6.8.
		0.5%	normal
0.25%	normal	0.25%	normal
control	normal	control	normal

Table 16

Dog, 12 kg.
Creatinine clearance with sustained infusion of a 0.1% creatinine solution in 0.85% saline.
Rate of infusion: 5 ml./min. from time zero onwards.
Infusion of 200 ml. 2% OES(DP 2000) from min. 240-285.

Period in min.	Clearance in ml./min.	Diuresis in ml./min.
180-210	135	0.47
210-240	133	0.47
infusion of OES		
240-285	140	0.31
285-315	113	0.37
315-345	103	0.66
345-375	114	0.70
375-405	115	1.00
405-435	127	0.66
435-465	136	0.66

Table 17

Dog, 10.6 kg.
Creatinine clearance with sustained infusion of a 0.1% creatinine solution in 0.85% saline.
Rate of infusion: 5 ml./min. from time zero onwards.
From min. 285-315 infusion of 200 ml. 4% OES(DP 300).

Period in min.	Clearance in ml./min.	Diuresis in ml./min.
0 min. start infusion as in previous experiment.		
230-255	106	0.64
255-285	103	0.66
infusion of OES		
315-345	93	0.80
345-375	63	1.00
375-405	38	1.33
405-435	38	1.40
435-465	80	1.60
465-495	73	1.60

colorimeter using the method of Folin and Wu (1919). Tables 16 and 17 show the figures for the creatinine clearance and the diuresis in two dogs, one infused with OES (DP 2,000), the other with OES (DP 300). In both experiments the animals received 200 ml. of a 2% and of a 4% solution. Although the infusion with OES (DP 2,000) did not change the clearance rate of creatinine the smaller molecule (OES DP 300) clearly caused a decrease in the renal creatinine clearance.

Effect of OES on monkey kidney cells in tissue culture

Cortical epithelial cells in primary culture were used and the cells cultured in Hanks medium (Hanks, 1949). Lactalbumen hydrolysate and calf serum in concentrations of 0.5% and 5.0% respectively were added. Two fractions of OES with DP's of 2,000 and 170 were used. The higher polymer fraction was dissolved in a concentration of 2% in Hanks medium to which was added 0.5% lactalbumin to give a viscous solution. Further dilutions of this were made to strengths of 1%, 0.5%, and 0.25%. A 4% solution of the lower-polymer fraction was prepared in the same medium as above and diluted to 2%, 1%, 0.5%, and 0.25%. The nutrient was then removed from the tissue cultures and replaced by the OES solutions. The cultures and the controls were examined 24 hr later (Table 18). It may be

Table 20

The influence of OES and Methylcellulose on the oxygen uptake of liver homogenates

No. of flasks	Medium (3.3 ml)	Q_{O_2} ml O_2/mg dried organ	Average Q_{O_2}
3	Krebs	7.7; 7.5; 7.1; 7.0; 6.9; 6.6; 6.2; 5.8	6.9
3	Krebs plus OES 2%(DP 170)	7.6; 7.4; 7.1; 7.0; 7.0; 6.9; 6.5; 6.1	6.9
3	Krebs plus OES 2%(DP 2000)	2.7; 3.6; 3.3; 3.2; 3.1; 3.1; 3.0; 2.9	3.1
3	Krebs plus methylcellulose 2%(DP 400)	1.3; 1.2; 1.3; 1.5; 1.3; 1.1; 1.3; 1.0	1.2

time (min.)	15	30	45	60	90	120	180	240

Table 19

Effect of dextran on monkey kidney cells in tissue culture.

Dextran DP 380

Conc. of dextran in medium	Results
4%	Some granular cytoplasm but cells still adherent to glass. pH, 6.1.
2%	normal. pH, 6.4.
1%	normal. pH, 6.6.
0.5%	normal. pH, 6.8.
0.25%	normal. pH, 6.8.

As the commercial dextran contains 6% glucose, this became metabolized by the tissue cells. The pH increased with decreasing glucose concentrations of the dextran.

noted that the greater viscosity of the higher polymer fraction damaged the tissue culture considerably. The same experiment was then carried out, using dextran solutions of different concentrations (Table 19). It is possible that the marked lowering of the pH of Hanks medium when added to the tissue cultures was due to the fact that the tissue cells metabolize the OES and, further, that the high viscosity of the high polymer fraction when added to the tissue culture, damaged and killed the cells.

Warburg experiments

To find out whether OES influences O_2 uptake in tissue cells, two fractions with DPs of 2,000 and 170 were tested on liver homogenates. The values were compared with those obtained from experiments with methylcellulose of a DP of 400 (Table 20). It was clearly shown that the lower fractions of OES with a DP of 170 had no effect on tissue respiration while OES with a DP of 2,000 diminished the O_2 uptake of liver cells. Methylcellulose (DP 500) had the same effect as OES 2,000. In addition, the oxygen uptake of liver homogenates, when in contact with two other high-polymer compounds, was measured; the values are given as percent of the normal

average oxygen uptake of controls. The average Q_{O_2} taken from 8 controls in Krebs medium was 7.5 ml. oxygen per mg. dried organ. Xylan-sulphuric acid ester with a DP of 100 decreased the oxygen uptake of the tissue cells by 25% and the cellulose-glycolic acid ether with a DP of 600 decreased the oxygen uptake by 10%. The cellulose-sulphuric acid ester was not tested because, as already stated, its toxicity is very high. It is evident that heteropolarity decreases the oxygen uptake of liver homogenates. Whether the viscosity of homopolar solutions or the shape of the molecules is responsible for the decrease in oxygen uptake cannot be said as yet.

Bacterial growth in OES solutions

In order to find out which bacteria would grow on OES solutions exposed to the open air for 7 days, aliquots of OES (DP 2,000) and OES (DP 170) were put onto trypsinized beef-agar plates. OES solutions with a DP of 2,000 and of 170 contained the following bacteria: Alcaligenes, Pseudomonas aeruginosa, Flavobacterium. A sample of hydrolyzed starch (DP 330) which was accidentally left uncovered for 14 days showed heavy growth of a species of micrococcus and yeasts. Staphylococcus aureus inoculated to a solution of OES (DP 2,000 and 170) grew rapidly.

Toxicity after long term administration of high polymers

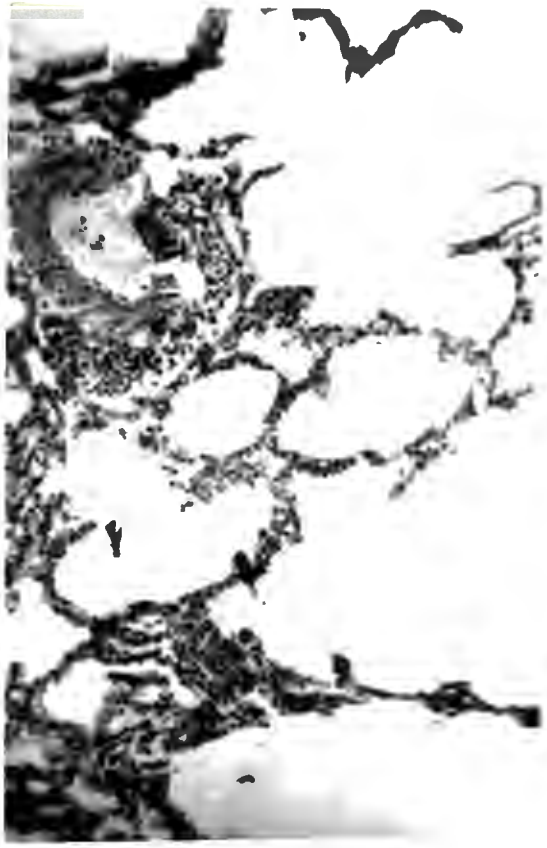
Some workers (Hodge, Maynard, Wilt, Blanchet, and Hyatt, 1950; Werthemann et al., 1951; Narat et al., 1952) have reported that methylcellulose, carboxymethylcellulose, polyvinylpyrrolidone, dextran, and xylan-sulphuric acid esters irritate various tissues. Giant cell tumours, vacuoles of reticulum cells, and foamy cytoplasm of Kupffer cells were found and xylan-sulphuric acid ester and dextran sulphate resulted in baldness and diarrhoea (Hirschboeck, Madison, and Pisciotta, 1954; Tudhope, Cohen, and Meikle, 1958). Ricketts, Walton, van Leuven, Birbeck, Brown, Kennedy, and Burt, however, did not find any toxic effects after the administration of dextran sulphate. This may be explained by the small dosage given (5,000 units) as against 40,000 - 90,000 units given by Hirschboek et al. (1954) and Tudhope et al. (1958).

It was expected that OES would show similar results and long-term toxicity experiments were set up. Rabbits, 2-3kg. in weight, were used; for 9 months they received 10 ml. of OES (DP 900) 3 times a week. No allergic reactions were observed during that period. No micro-photographs of the various organs of treated and control animals can be shown as the slides were lost when given to the Department of Pathology for comment on the sections.

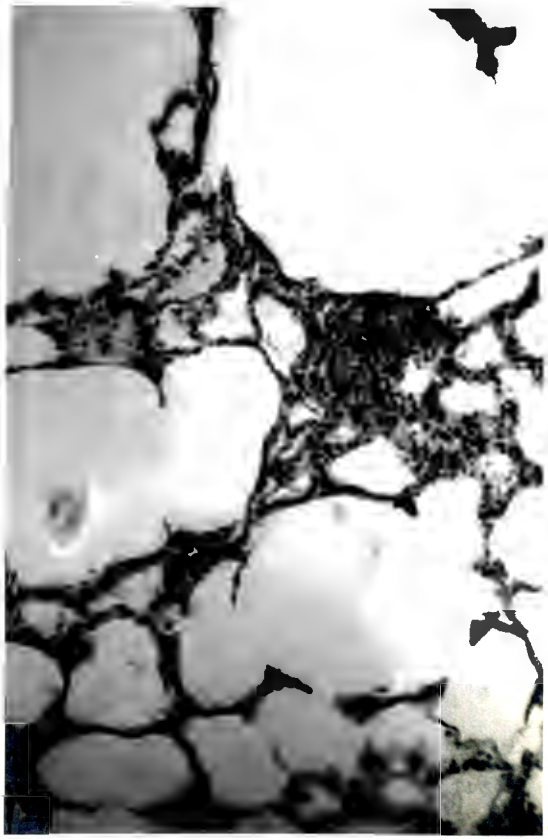
According to the member of the department who inspected the slides, no marked changes in liver, kidney, spleen, or lung were observed, but there was slight congestion of the lung tissue and vacuoles were found in the reticulum cells of the liver. As injections of polysaccharides often cause quite unspecific changes in tissues, such as congestion and swelling (Hetzel, 1952), OES did not differ significantly from other polysaccharides in this respect. These unspecific reactions of tissue after injection of polysaccharide have given rise to much controversy, and publications continue to appear both in favour of and against the statement that high polymers cause definite alterations in tissues.

The same slight changes as mentioned above were observed in tissue slides taken from rabbits which received 10ml. of 6% dextran intravenously twice weekly for 8 weeks. Liver and spleen showed slight congestion, but lung and kidney were unchanged as compared with the controls.

Intraperitoneal injection of OES into rats, 120-160g. in weight, gave similar results. The animals were injected 3 times weekly with 4ml. of OES (DP 2,000 and 170) in concentrations of 2% and 4% respectively. Liver and spleen showed slight swelling and the lungs were deformed by multiple abscesses. Microscopically the



(a)



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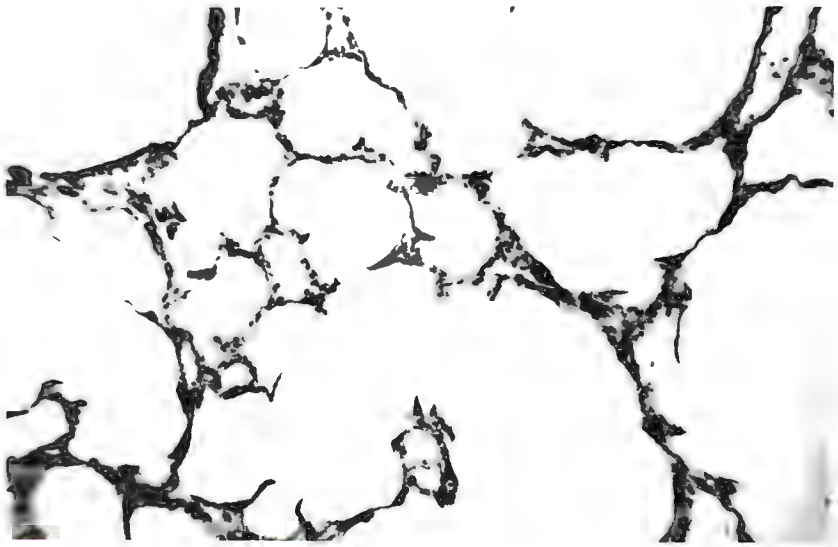
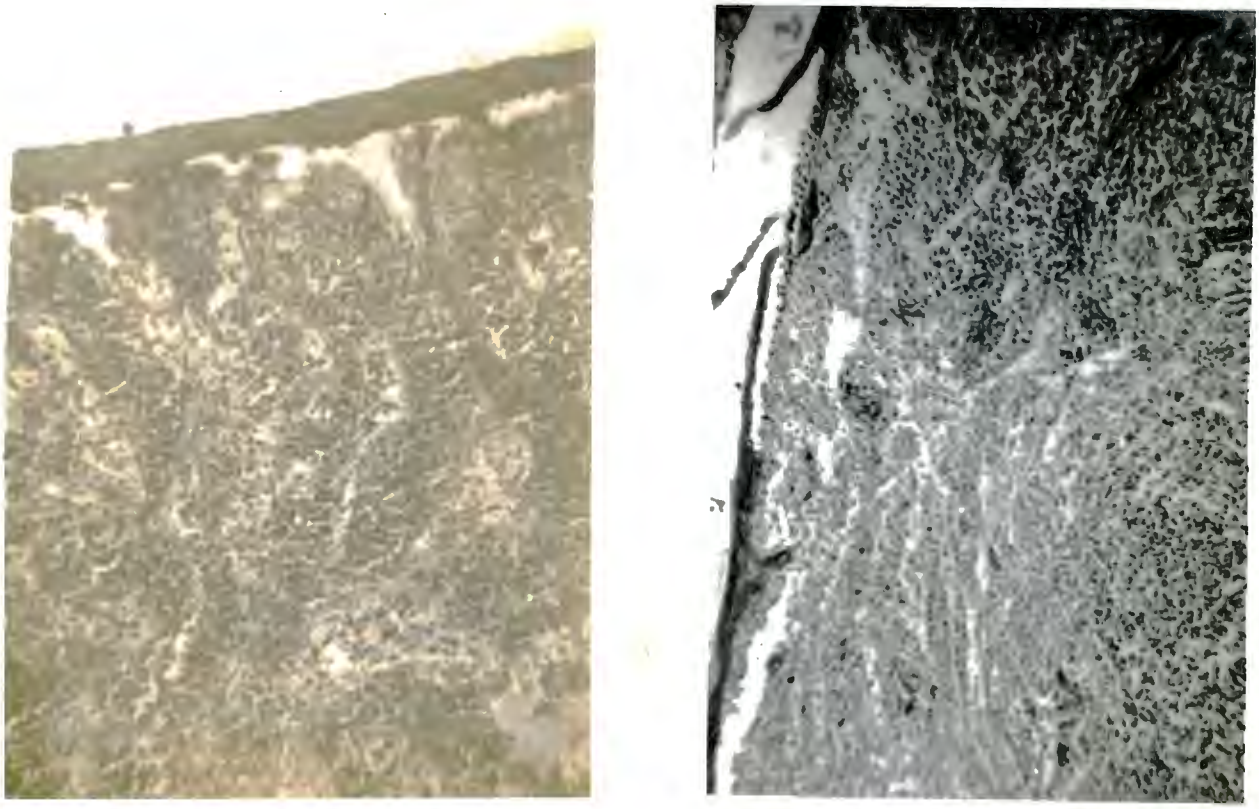
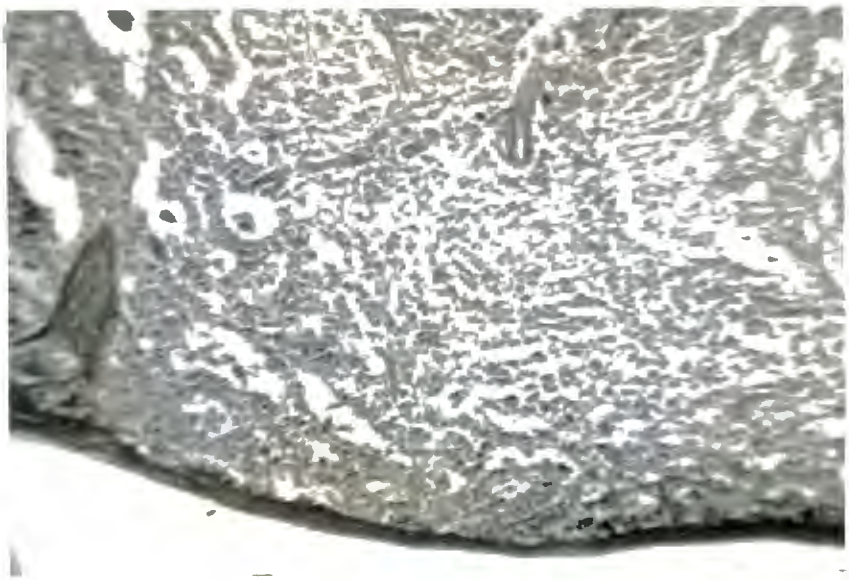


(c)

Fig.20 Microphotographs of Rabbit Lungs
(Stained with P A S. All magnifications × 120)
(a) Injected with OES 3% DP 2000
(b) Injected with OES 3% DP 170
(c) Control



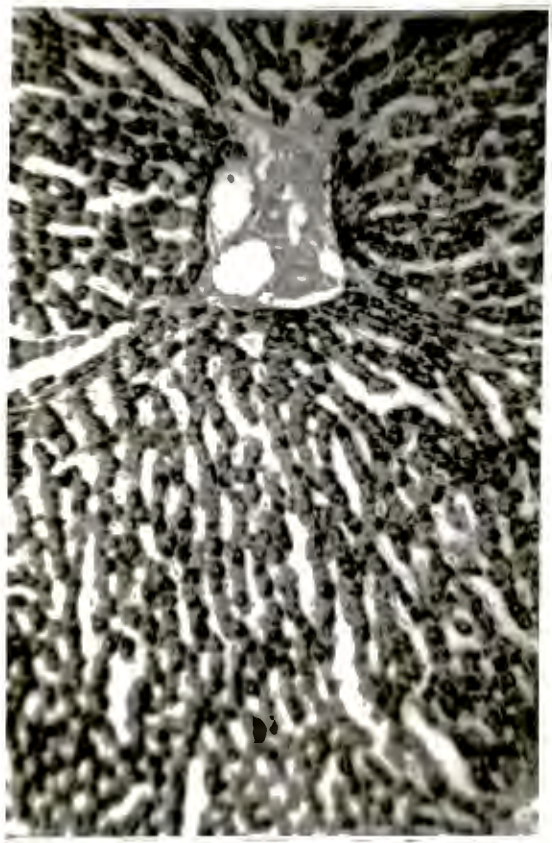
(a) (b)



(c)

Fig.1@ Microphotographs of Rabbit Spleens

(Stained with P A S. All magnifications x 120)
(a) Injected with OES 3% DP 2000
(b) Injected with OES 3% DP 170
(c) Control



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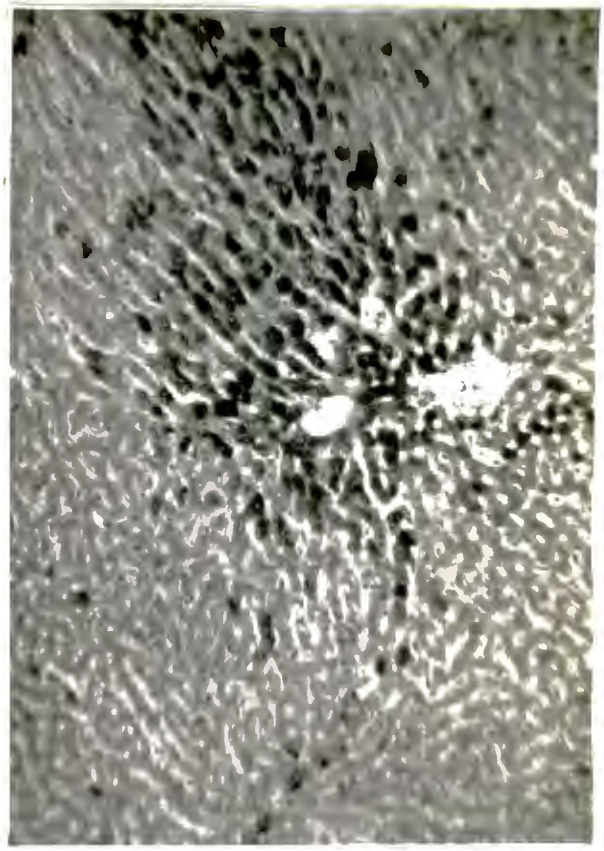
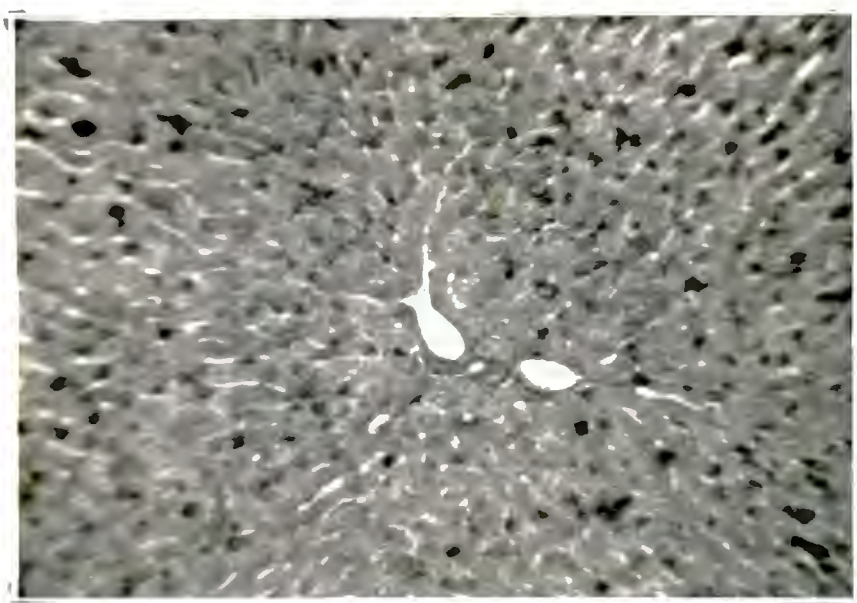


(b)



(c)

Fig.19 Microphotographs of Rabbit Livers

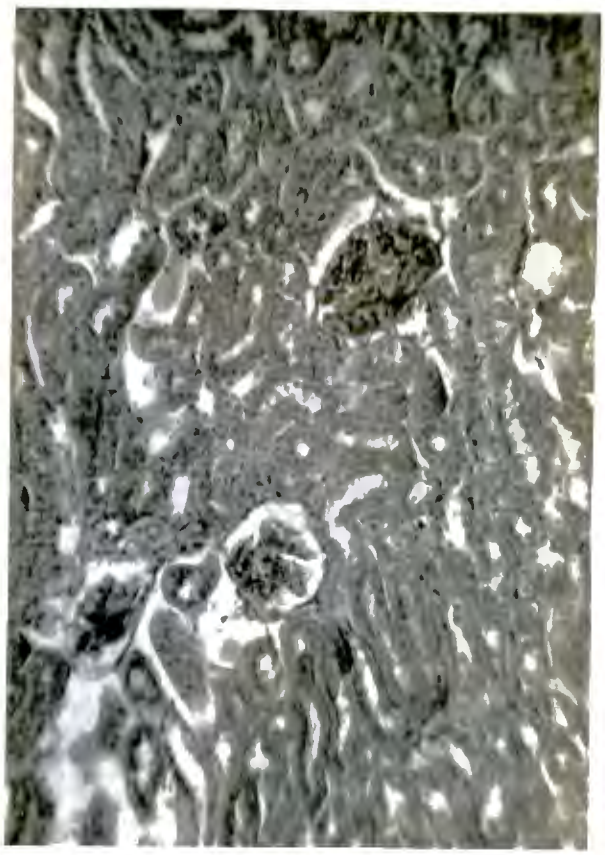
(Stained with P A S. All magnifications x 120)

(a) Injected with OES 3% DP 2000

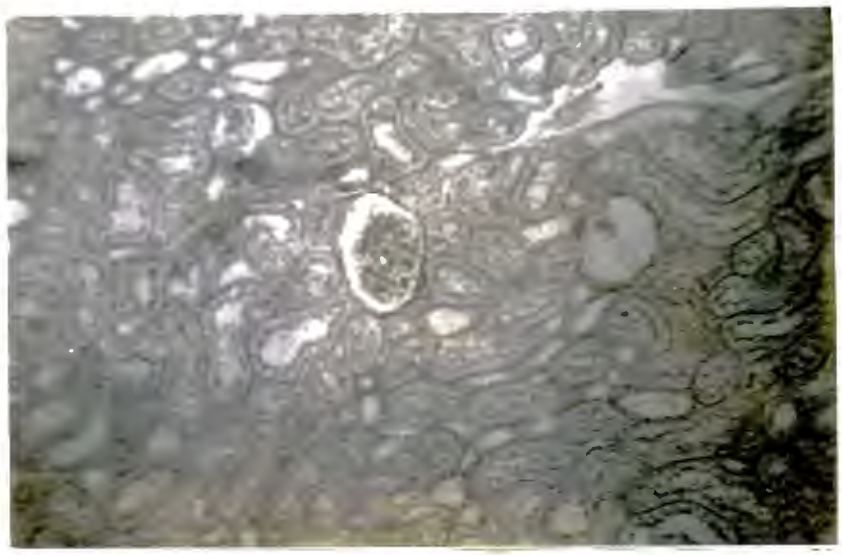
(b) Injected with OES 3% DP 170

(c) Control

(a)



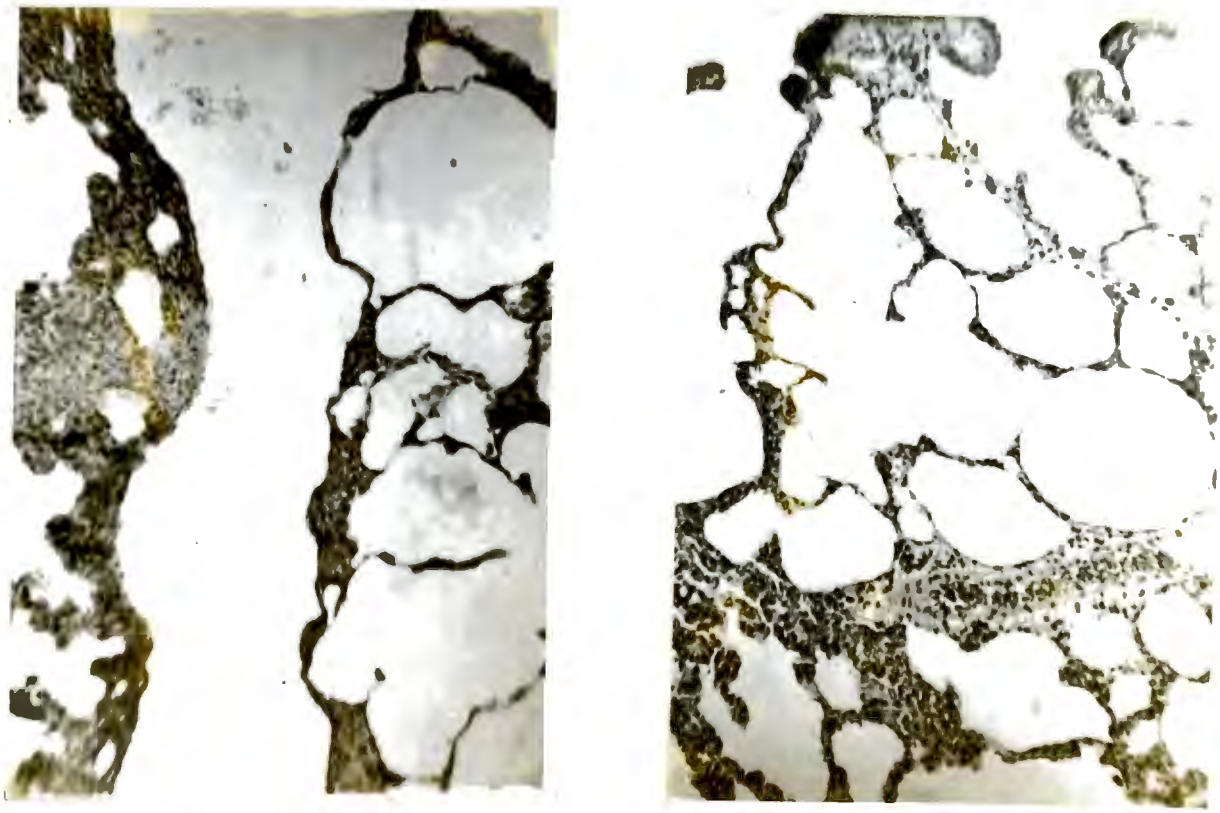
(b)



(c)

Fig.17 Microphotographs of Rabbit Kidneys

(Stained with P A S. All magnifications x 120)
(a) Injected with OES 3% DP 2000
(b) Injected with OES 3% DP 170
(c) Control



(a) (b)

Fig.16

Microphotographs of Rat Lungs

(Stained with H.and E. All magnifications x 120)
(a) Injected with OES 2% DP 2000
(b) Control

remaining functional lung tissue was emphysematous. The same, however, could be seen in some of the control rats which also showed abscesses in the lungs. As the changes in the different tissues examined were not very marked, the microphotographs were of no help in establishing clearly the slight alterations in the tissues. The lung tissues of a rat injected with OES and of a control are shown in Fig. 16.

In order to show the possible effects (e.g. of storage) of different fractions of OES on animal tissues, 4 rabbits of 1.8-2.2kg. were used. Three times a week, 2 rabbits received 10ml.of a 3% solution of OES (DP 2,000) and 2 rabbits the same dose and amount of OES (DP 170). Three weeks later, all 4 rabbits were killed 18 hours after the last injection and the lungs, livers, spleens, and kidneys were placed into alcoholic picroformol and stained with Periodic Acid Schiff (P.A.S.) (Figs. 17-20). These microphotographs indicate that OES of a high DP is deposited more extensively in the liver, kidney, and spleen than OES with a low DP. The microphotographs of lung tissue, however, do not show marked differences in the storage of OES, DP 2,000 and OES, DP 170. But there is a marked difference between these and the control.

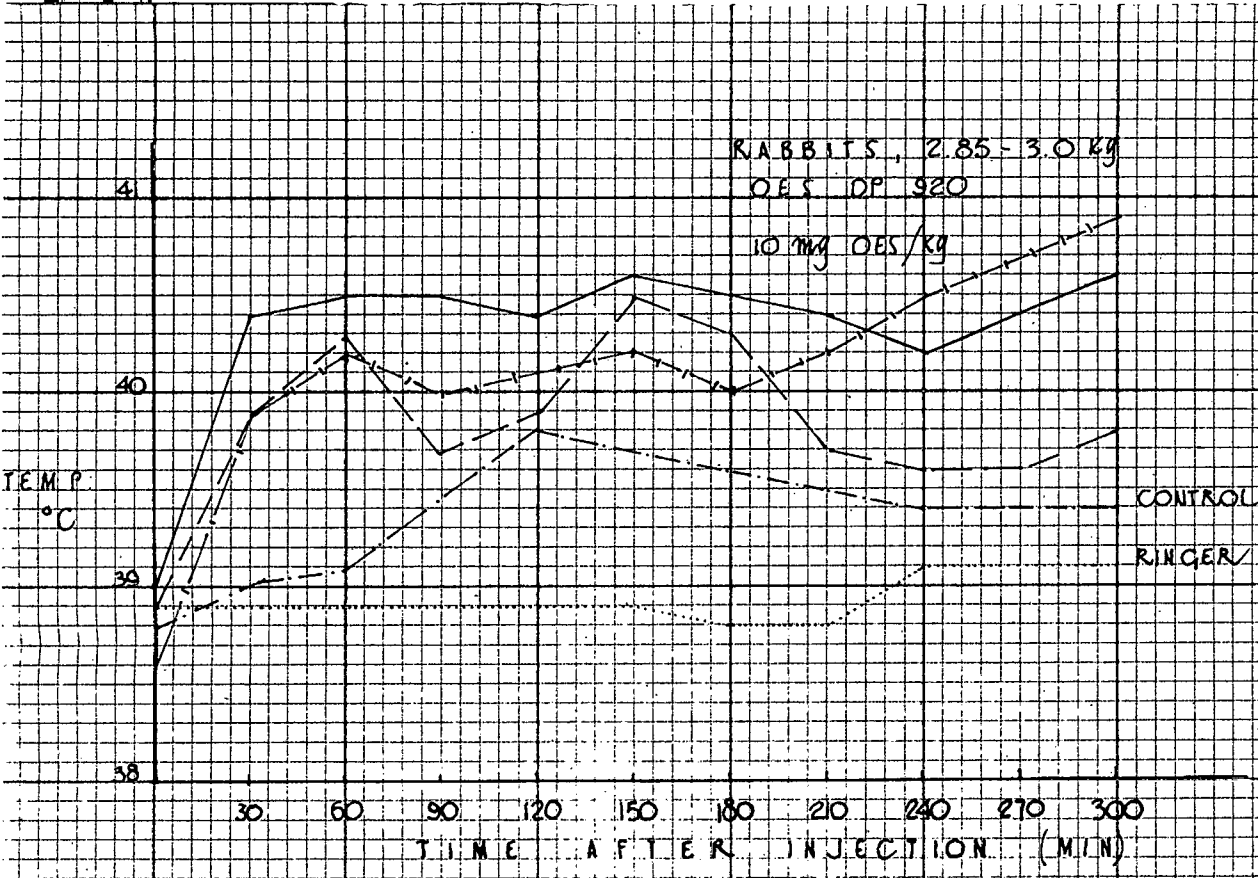


Fig.24

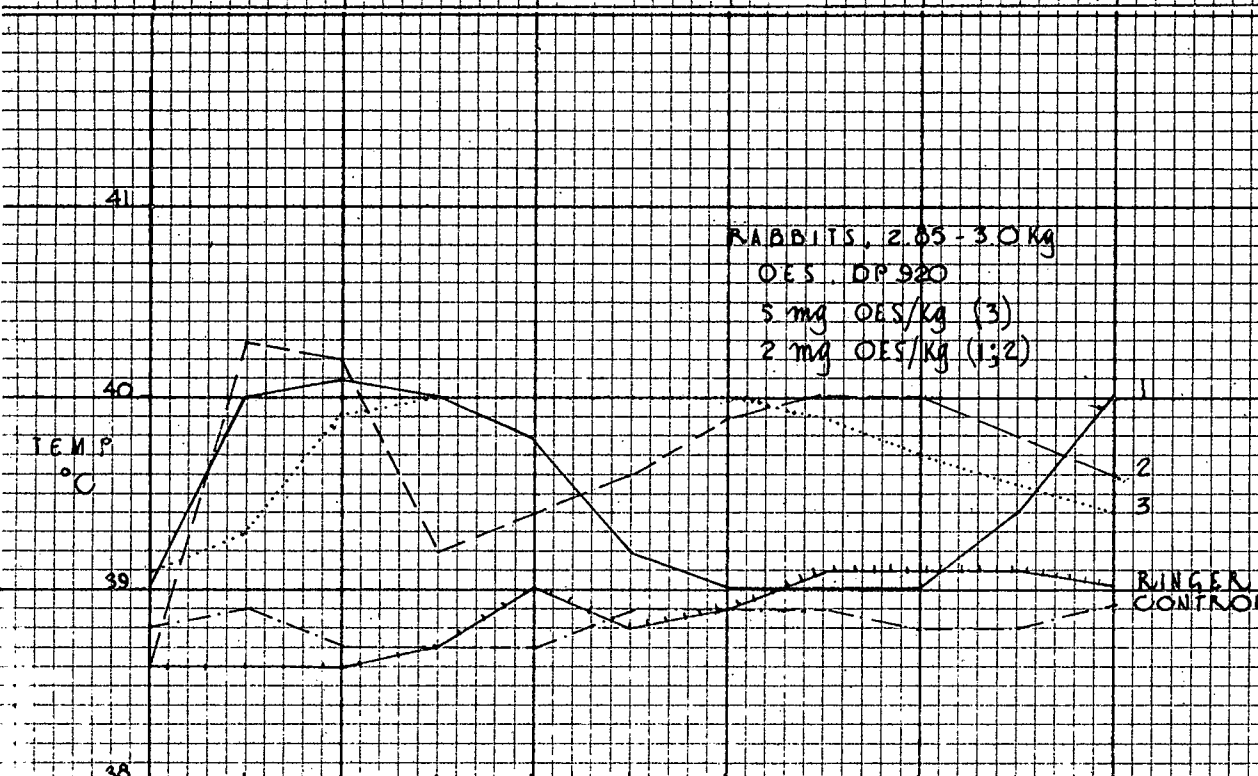


Fig.24 (continued)

Effect of injection of OES on the body temperature

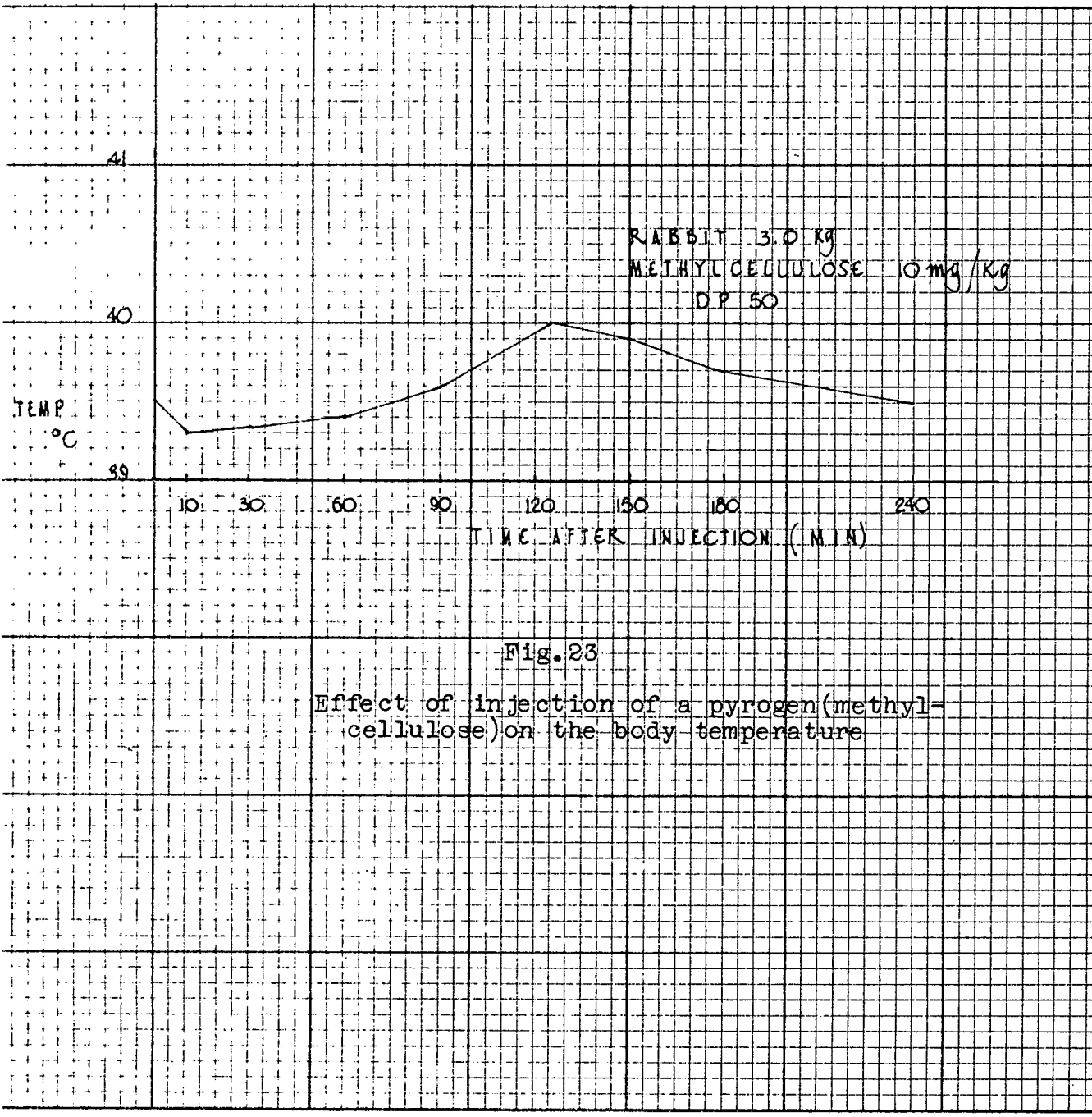


Fig. 23

Effect of injection of a pyrogen (methyl-cellulose) on the body temperature

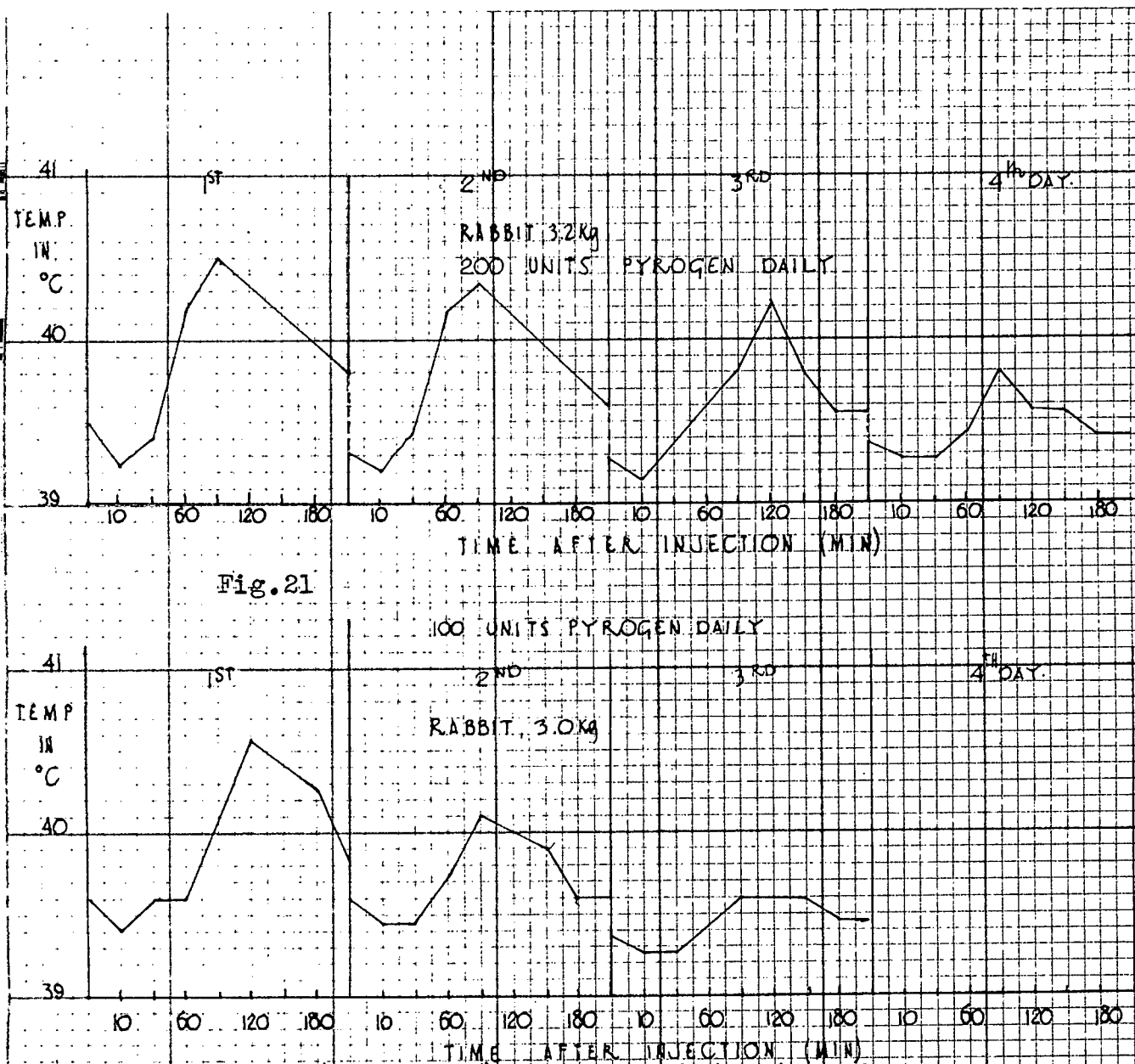


Fig.21

Fig.21(continued) Effect of injection of a pyrogen (Pyrifer) on the body temperature

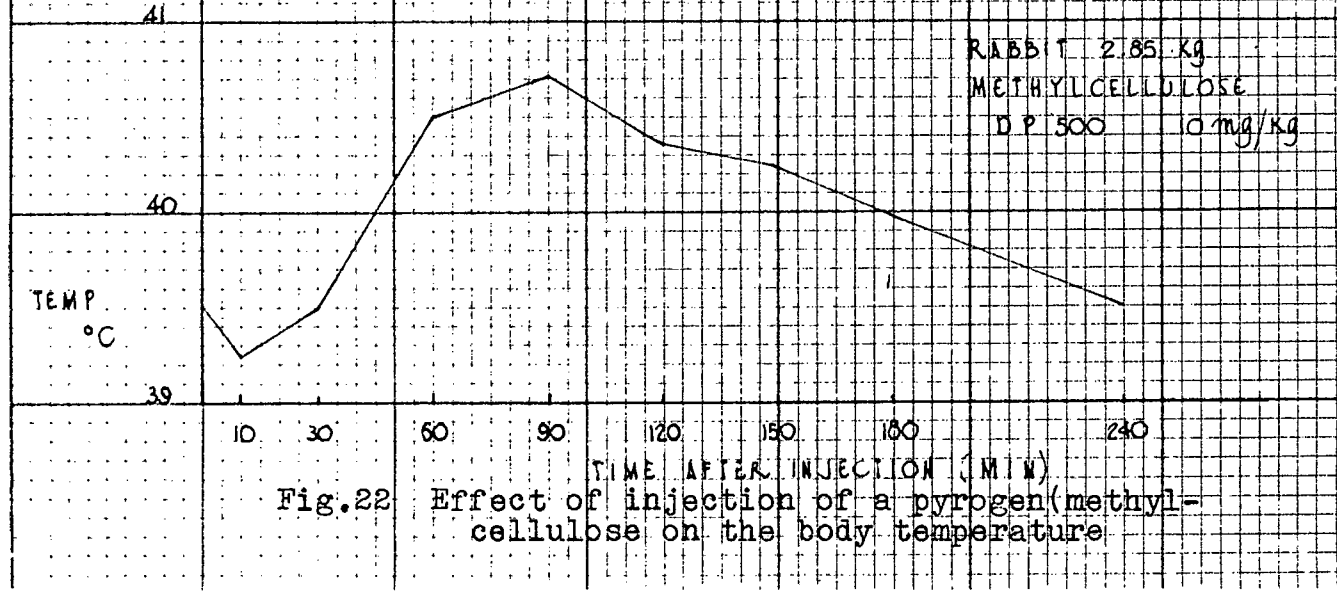


Fig.22 Effect of injection of a pyrogen(methyl-cellulose on the body temperature

The influence of OES on body temperature

Several polysaccharides of various degrees of polymerisation and polarity were investigated and it was found that methylcellulose caused an increase in body temperature, unlike suspensions of bacteria which must be injected in higher and higher concentrations to achieve the same effect (Bruns, Hahn, and Schild, 1950; Bennet and Beeson, 1953; Wood, 1958). On the other hand, equal doses of methylcellulose may be injected for many weeks with a resulting rise in temperature each time. This fact was made use of in testing antipyretic drugs (Enders, Hertlein, and Wiedersheim, 1953). Repeated investigations of the antipyretic action of different drugs on the same animal can thus only be made with a pyrogen such as methylcellulose, which causes the same rise in temperature with each injection and evokes no tachyphylactic response from the animal. Figs. 21-23 show the comparison between repeated doses of a pyrogenic substance and methylcellulose.

OES (DP 920), in various concentrations, was injected into rabbits intravenously and the temperature taken rectally. There was an immediate rise of the body temperature which lasted for 1-1½ hours (Fig. 24).

In order to ascertain whether the rise in temperature was caused by the linear amylose (DP 470) only or by the more complex molecule of amylopectin (DP 935), these

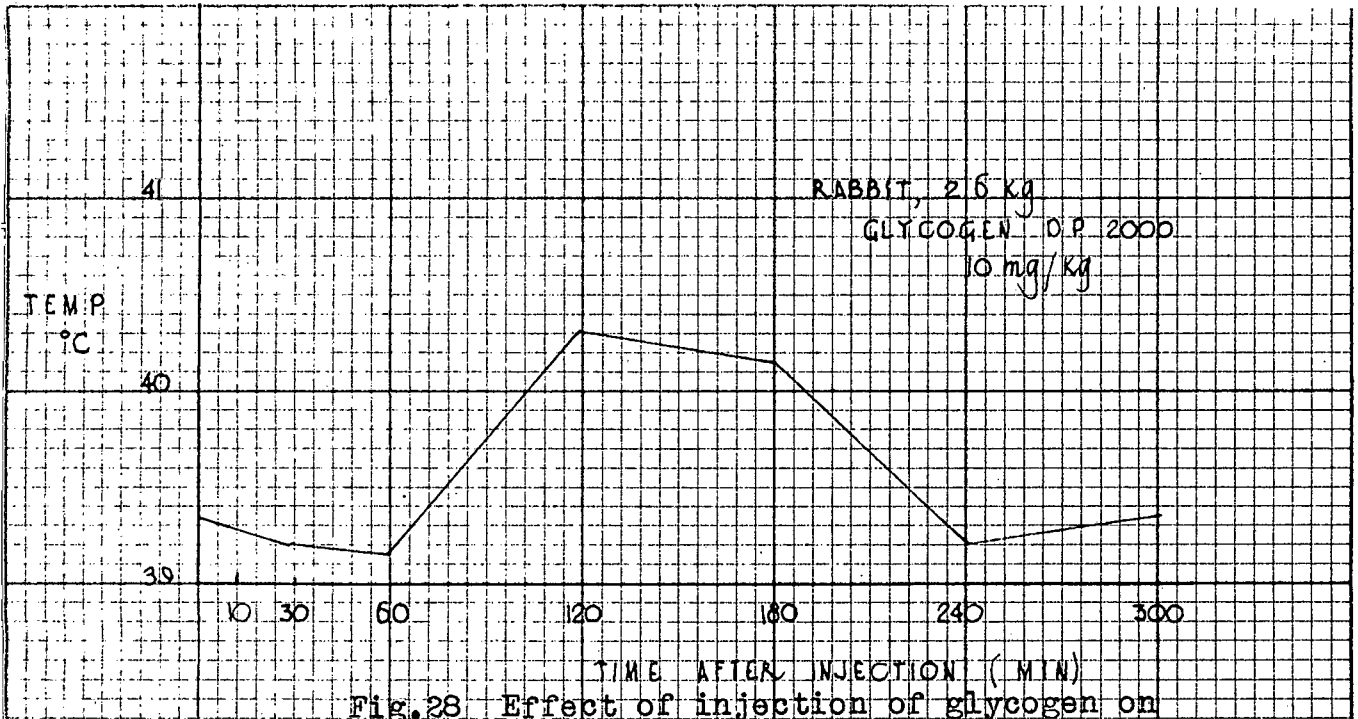


Fig.28 Effect of injection of glycogen on the body temperature

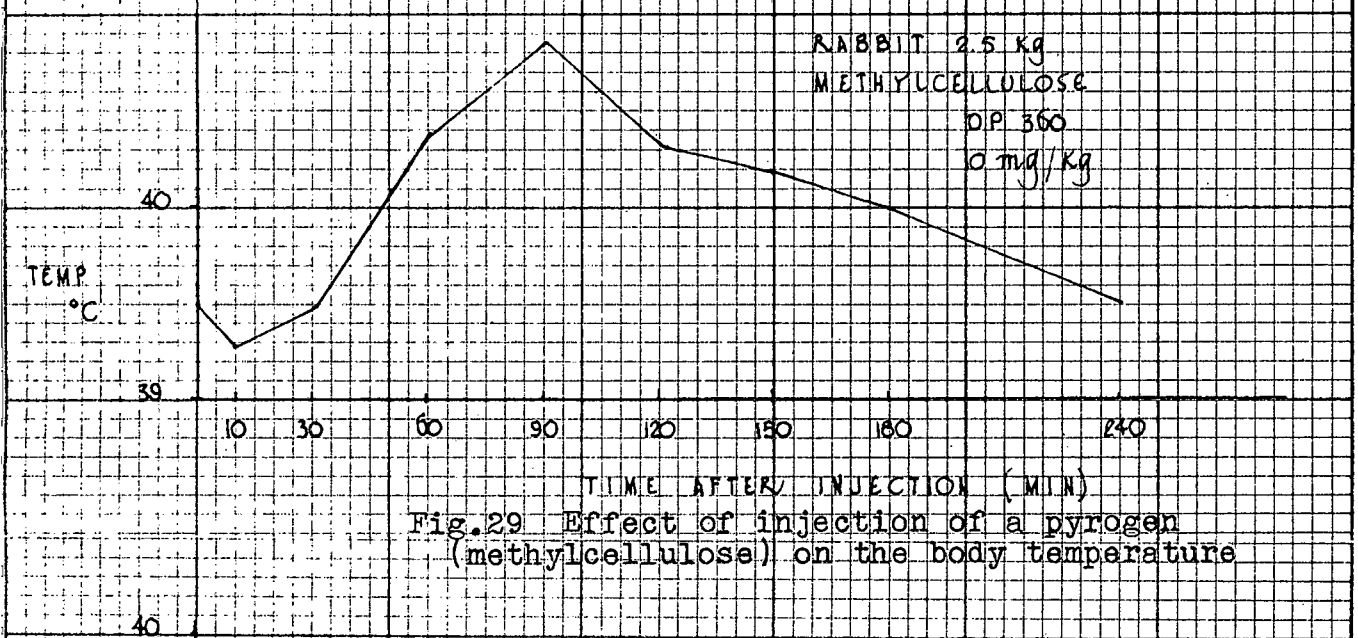


Fig.29 Effect of injection of a pyrogen (methylcellulose) on the body temperature

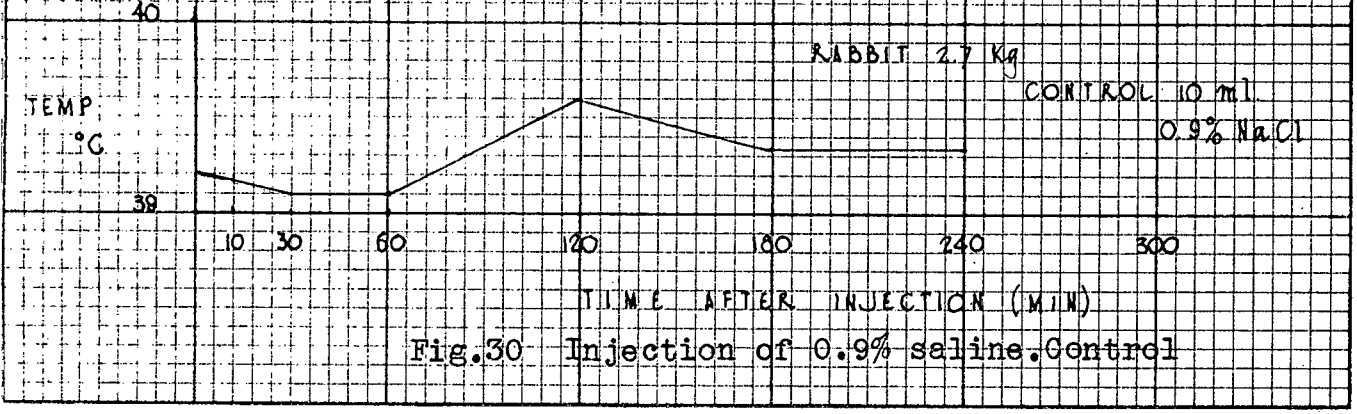


Fig.30 Injection of 0.9% saline. Control

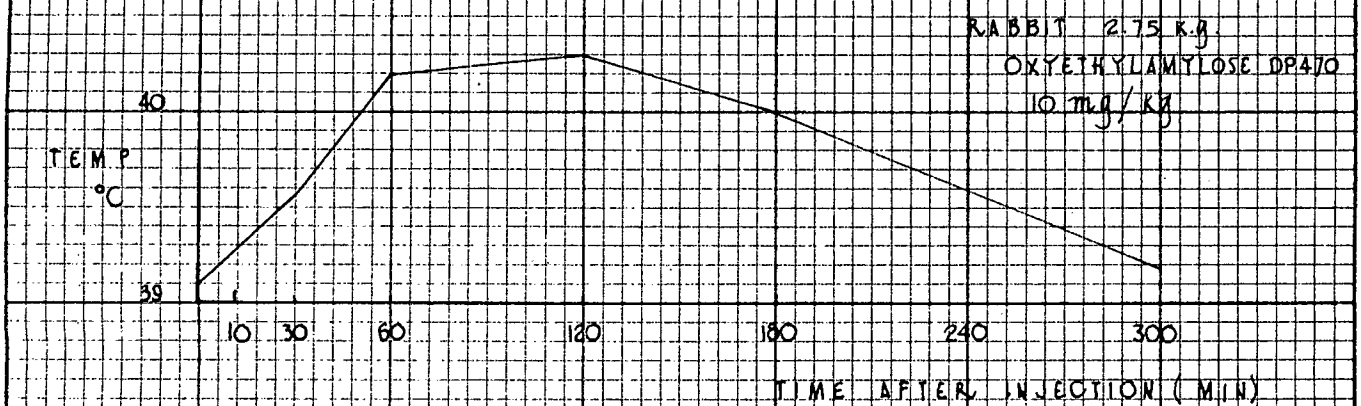


Fig.25 Effect of injection of oxyethylamylose on the body temperature

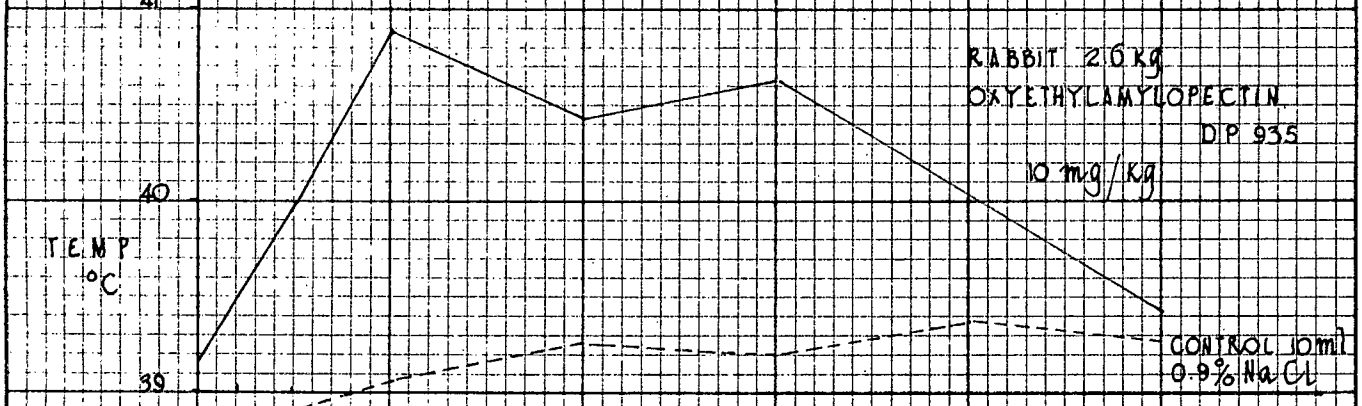


Fig.26 Effect of injection of oxyethylamylopectin on the body temperature

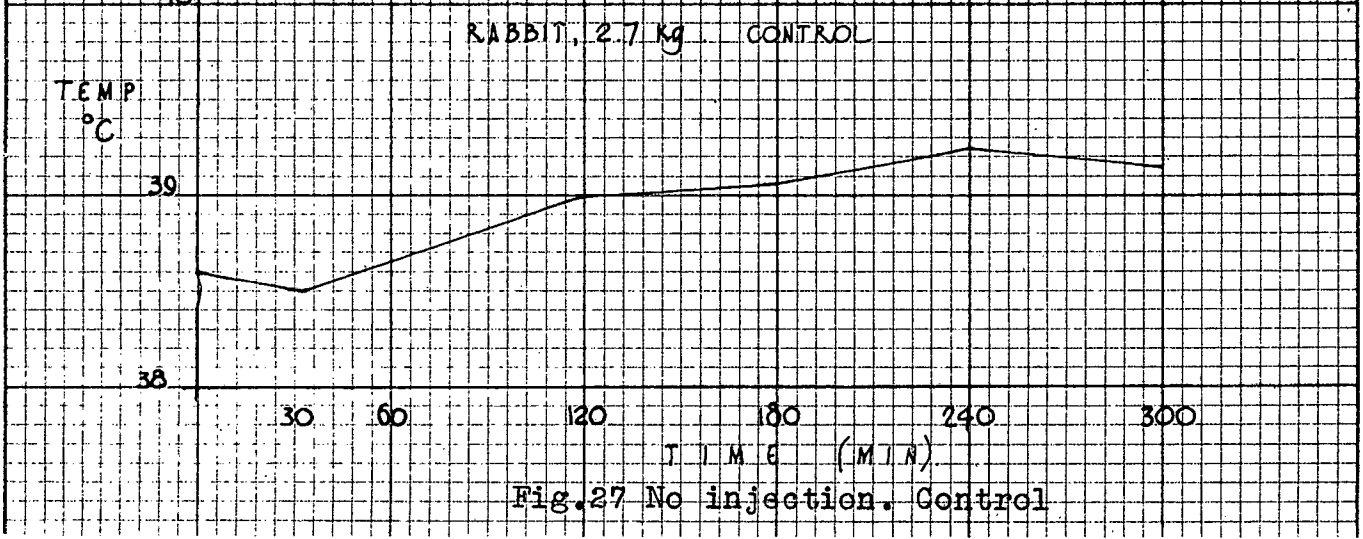


Fig.27 No injection. Control

fractions were injected separately and the effect on temperature recorded (Figs. 25-27). In both cases a rise in temperature was recorded.

As the branched molecules of amylopectin cause a rise in body temperature, it was expected that the spherical molecules of glycogen would similarly influence the body temperature. Figs. 28 and 29 show the action of glycogen and methylcellulose in raising the body temperature. The control to these experiments is given in Fig. 30.

The effect of OES on the white blood cell count

It has been reported that high polymer substances when injected intravenously or intraperitoneally alter the ratio of neutrophils to lymphocytes and cause a leucopenia which is sometimes followed by a reactive lymphocytosis. The high polymers used were mainly water-soluble cellulose derivatives (Bucher and Staub, 1940; Staub and Bucher, 1943; Wiedersheim et al., 1953) or polysaccharides from bacteria (Poel and Belkin, 1952). In most of the experiments the intravenous injection caused a leucopenia with a relative increase in lymphocytes and a subsequent leucocytosis. Some workers state that after intraperitoneal injection of 2 - 3 ml.of bacterial polysaccharides, leucopenia can be observed after 1 hour and lasts for 4 - 6 hours (Poel, 1951).

Autotransfusion of labelled white cells causes a retention of leucocytes in the lung and a consequent decrease in the white cells of the peripheral blood and this lasts for 4-6 hours (Weisberger, Storaasli and Hannah, 1950). There are different views as to why these changes in the physiological distribution of the white cells take place: the mechanical retention of white cells, usually the large one, due to the partial blocking of the capillaries in the lung, is said to be the reason for the leucopenia (Bucher et al., 1940; Bierman, Kelly, King and Petrakis, 1951.) On the other hand Bierman, Kelly, Petrakis, Cordes, Foster and Lose (1951) and Weisberger, Guyton, Heinle and Storaasli (1951) consider it to be due to the presence of histamine or to some specific activity of the lung.

The rouleaux formation of red cells and their blocking of lung capillaries or the stimulation of the ACTH mechanism (Recant, Hume, Forsham and Thorn, 1950) with its influence upon distribution and release of white cells, are also considered to cause the onset of leucopenia (Faludi, 1938; Dalton and Selye, 1939; Bierman, Kelly, Cordes, Byron, Polhemus and Rappoport, 1952; Gabrilove, Volterra, Jacobs and Soffer, 1949). The size and shape of the high polymers has little, if any, influence upon the retention of white cells in lung

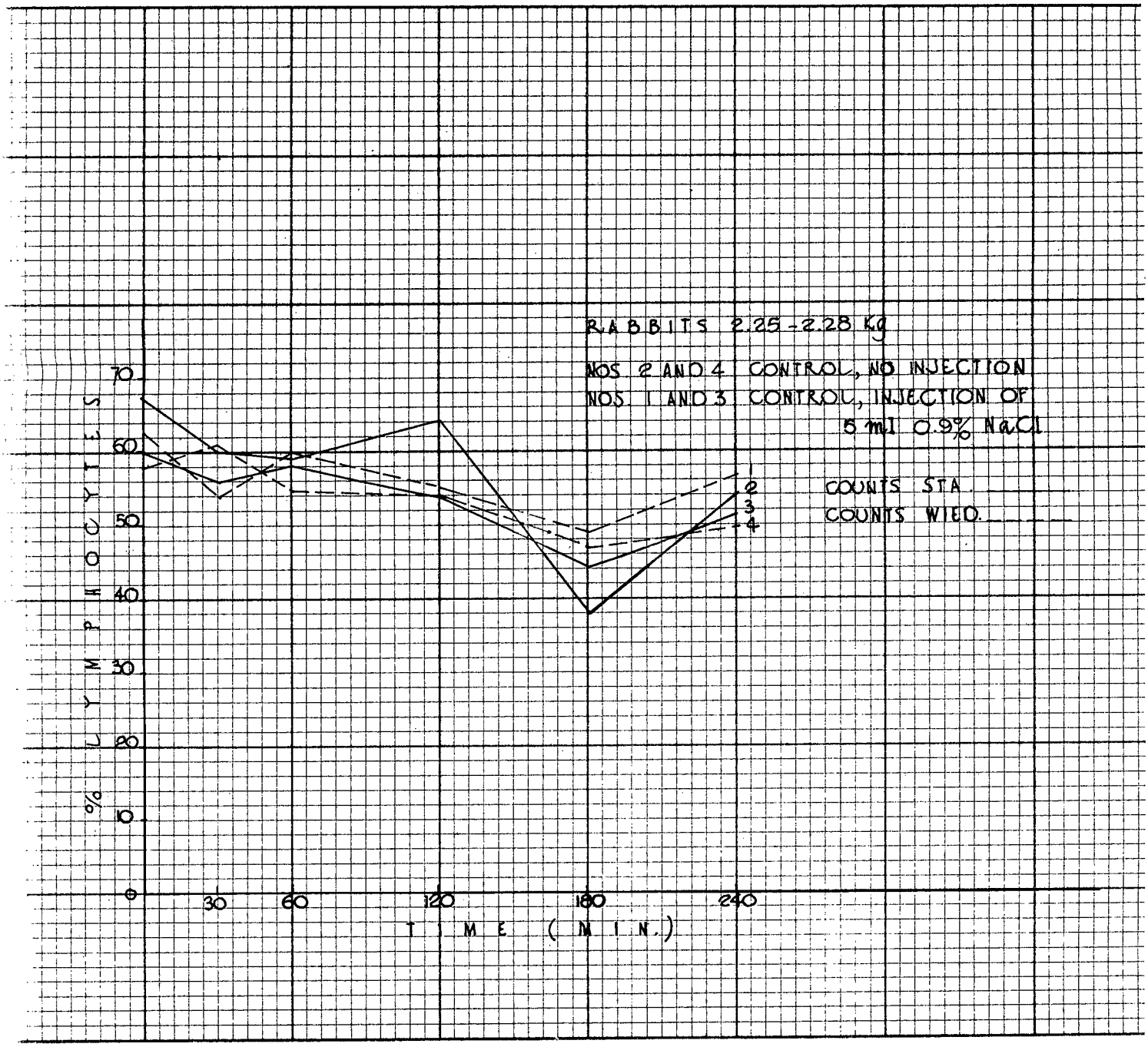


Fig.31

Two comparative counts of lymphocytes (differential count), no injection, control and two counts after injection of 0.9% saline, control

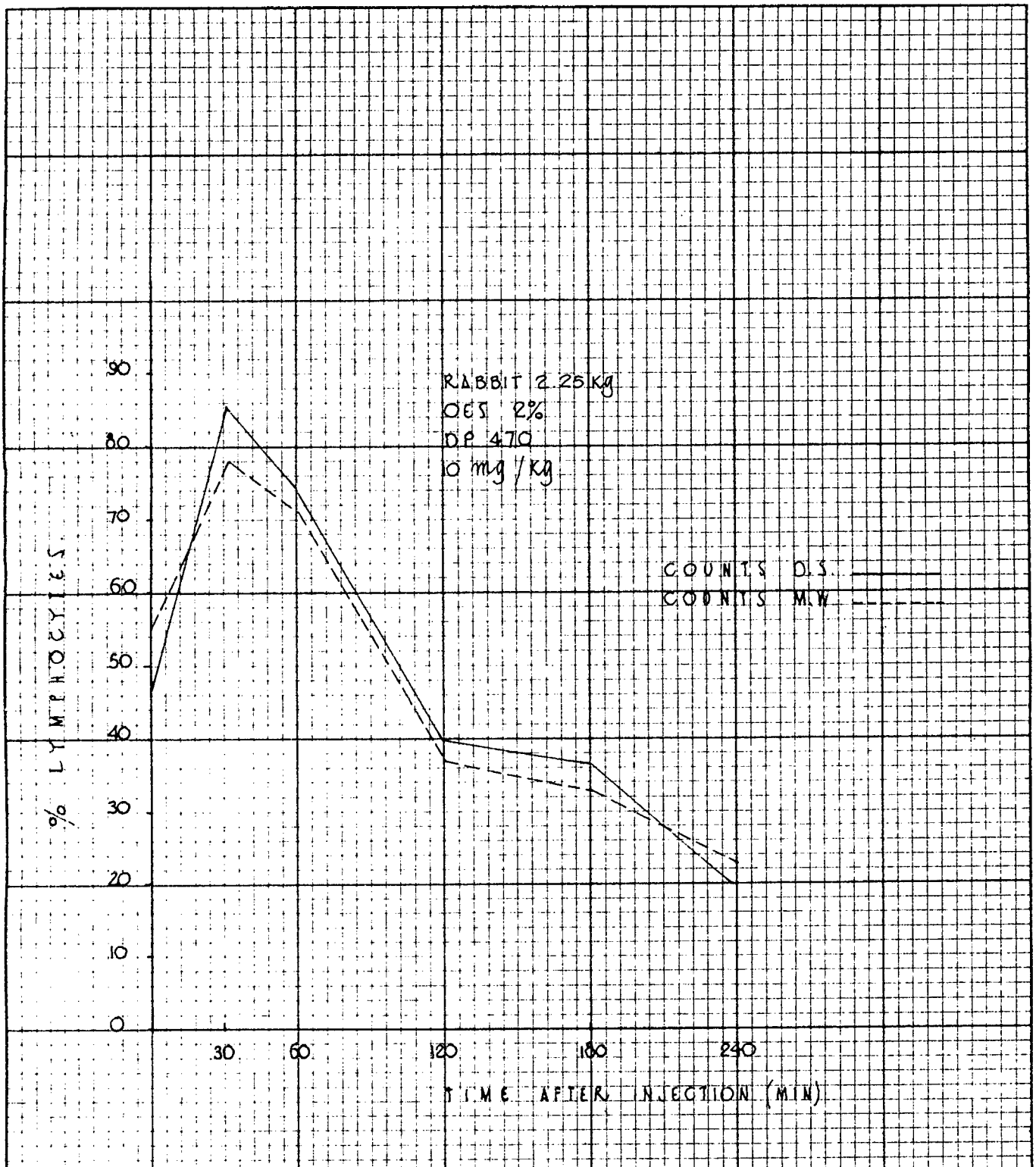


Fig.31

Two comparative counts of effect of OES on lymphocytes (differential count)

Table 21

Heart-Lung preparations. Nembutal anaesthesia.

	Total white cell count in c.mm.		
Dog, 12.5 kg. 20 ml.3% OES DP 920	1	2	3
	6700	4800	2200
Dog, 9.8 kg. 20 ml.3% OES DP 170	1	2	3
	9000	6800	4300
Dog, 8.7 kg. 20 ml.3% methylcellulose DP 360	1	2	3
	7300	5000	1800

The numbers 1-3 represent:

1, white cell count before the operation

2, white cell count with heart-lung preparation working

3, white cell count after addition of test substance to heart-lung preparation

capillaries (Wiedersheim, 1953) but the polarity of the substances changes the number of white cells in the blood. The heart-lung preparation shows that high polymers added to the blood cause a leucopenia (Table 21) and this is an important factor in explaining the mechanical retention of white cells in the lung.

The effect of OES on the white blood cells was demonstrated using high, medium, and low fractions with degrees of polymerisation of 2,000, 920, 470, and 170 respectively. The animals used were rabbits of no special breed or sex. The concentration of OES injected was 1-2% for the high polymer fraction and 4% for the medium and low fractions. It is known that the white blood cell count of rabbits is affected very much by emotional stress (Nice and Katz, 1936; Hueper, Landsberg and Eskridge, 1940) and considerable care was taken to count accurately and the results were compared with those obtained independently by another member of the staff. The results showed close agreement (Fig. 31). The normal white cell counts of the rabbits used in these experiments were compared with those reported by other workers (Cheng, 1930; Casey, Rosahn, Hu and Pearce, 1936; Casey, 1940; Scott, Richards and Loh, 1940; Sturgis and Bethell, 1943; Wintrobe, 1956. Scarborough (1931) found 7,900 white cells with 41.8% lymphocytes. These workers

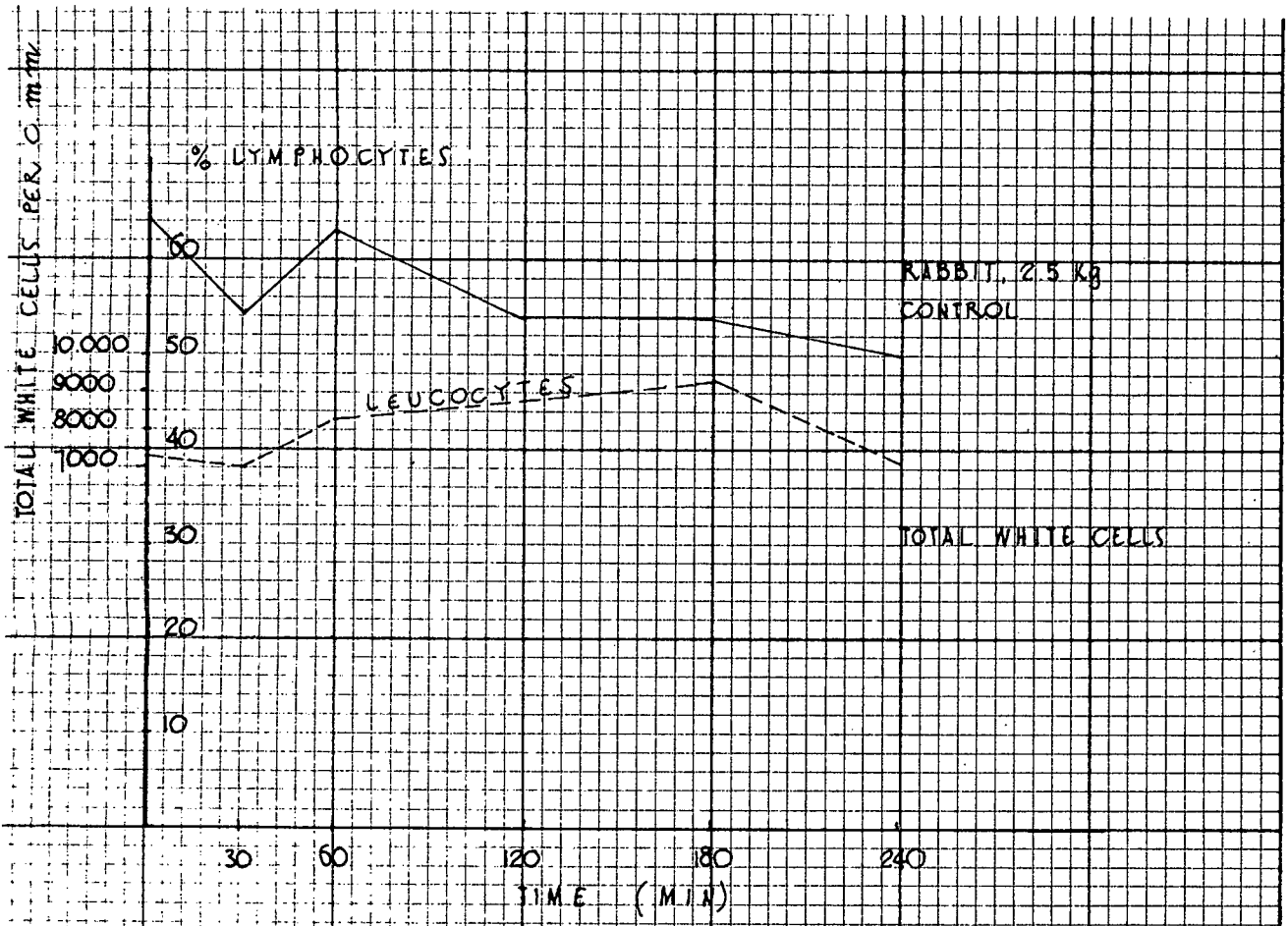


Fig.36 White blood cell count and percentage of lymphocytes, no injection. Control

Fig.37 White blood cell count and percentage of lymphocytes after injection of 0.9% saline. Control

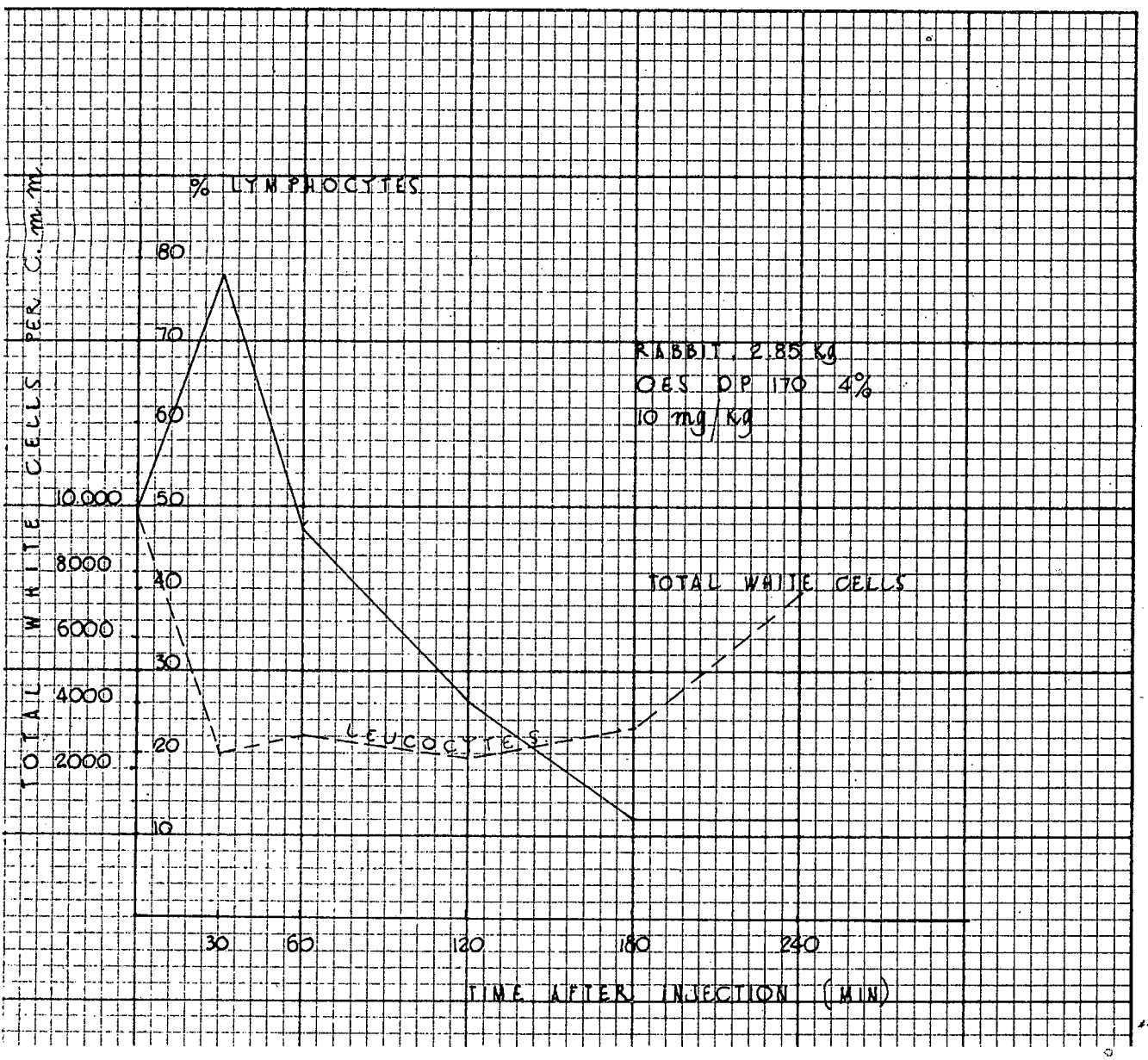


Fig. 35

The effect of injection of OES on the total white cell count and on the percentage of lymphocytes.

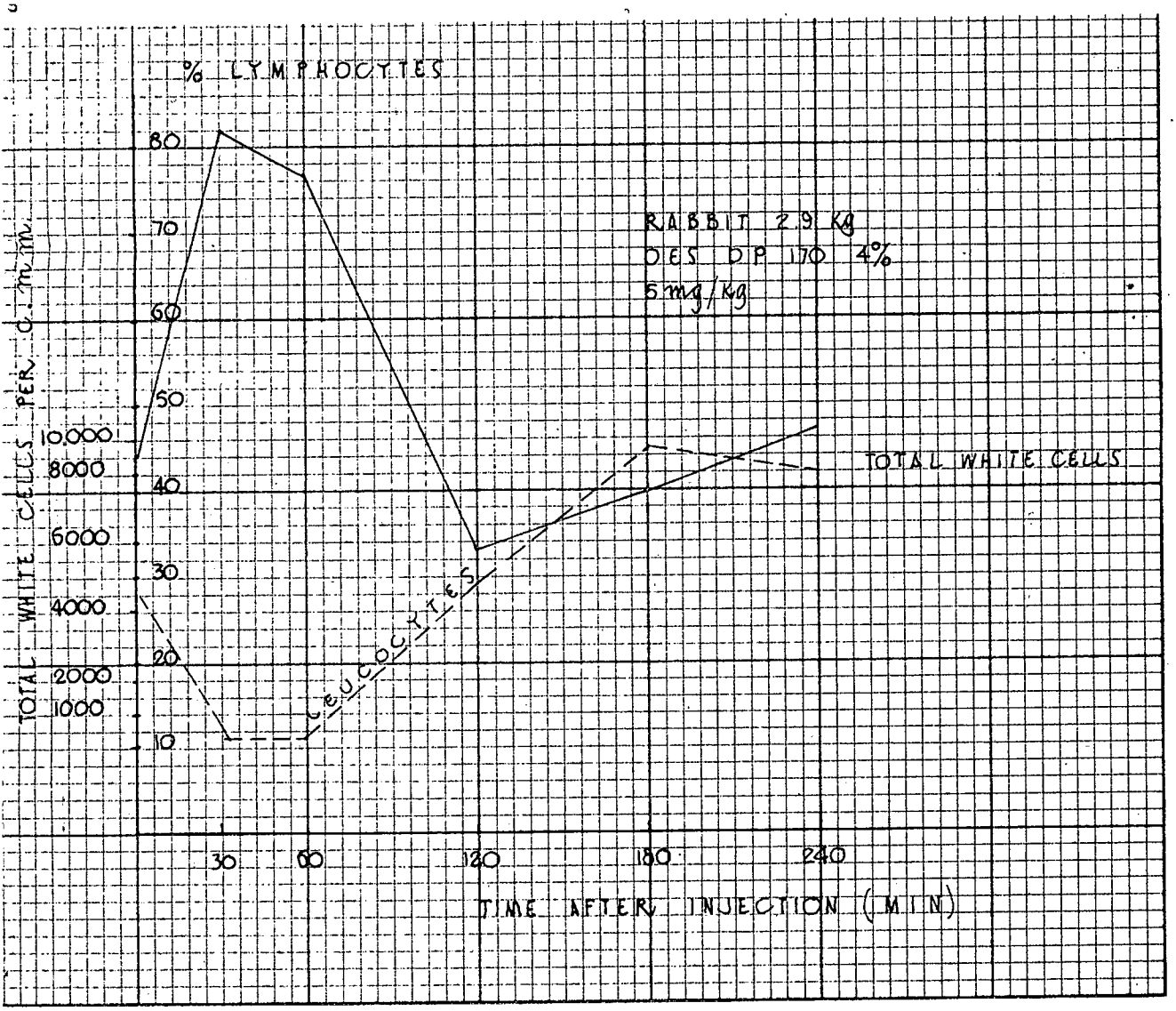


Fig. 35

The effect of injection of OES on the total white cell count and on the percentage of lymphocytes.

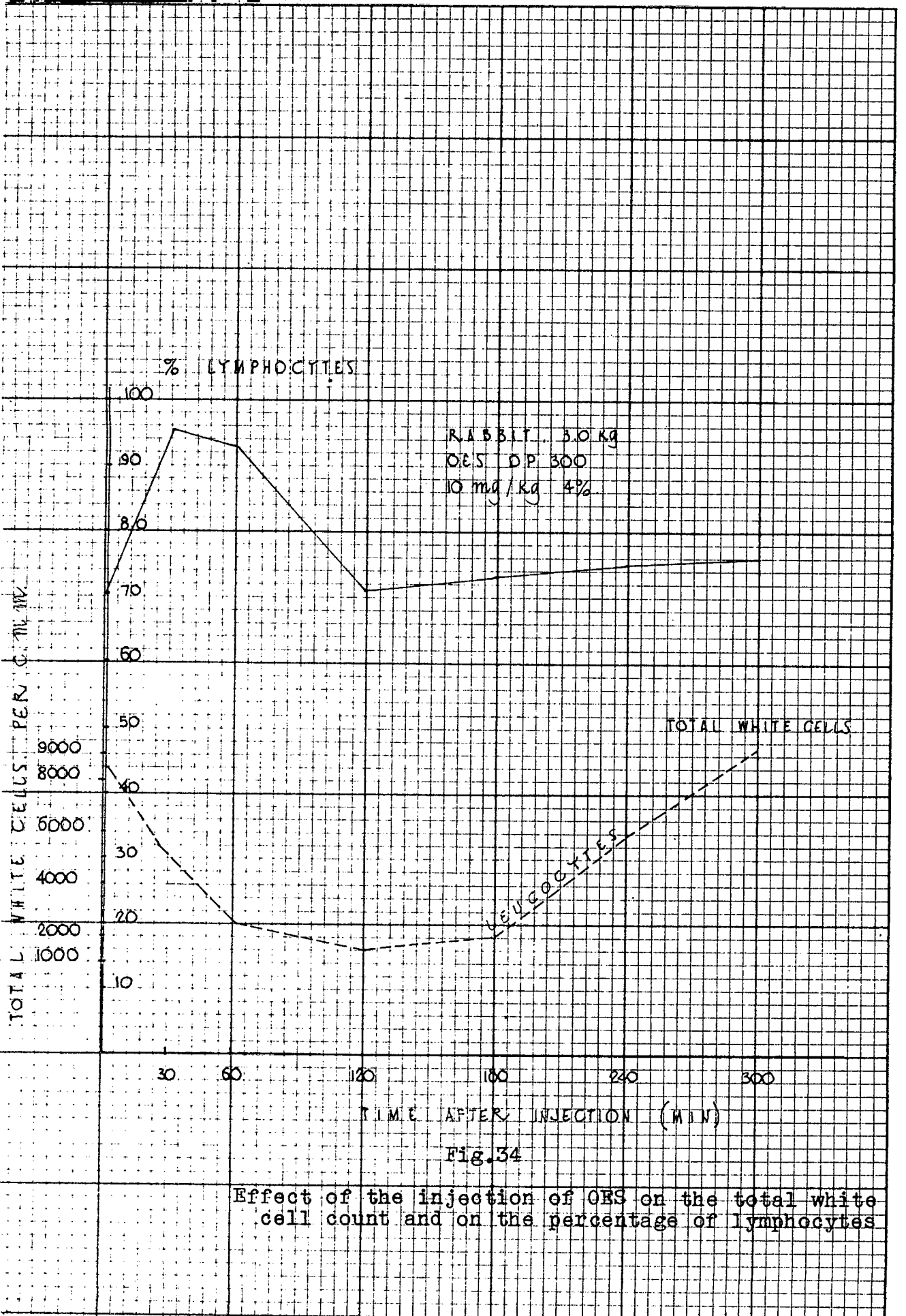


Fig.34

Effect of the injection of OES on the total white cell count and on the percentage of lymphocytes

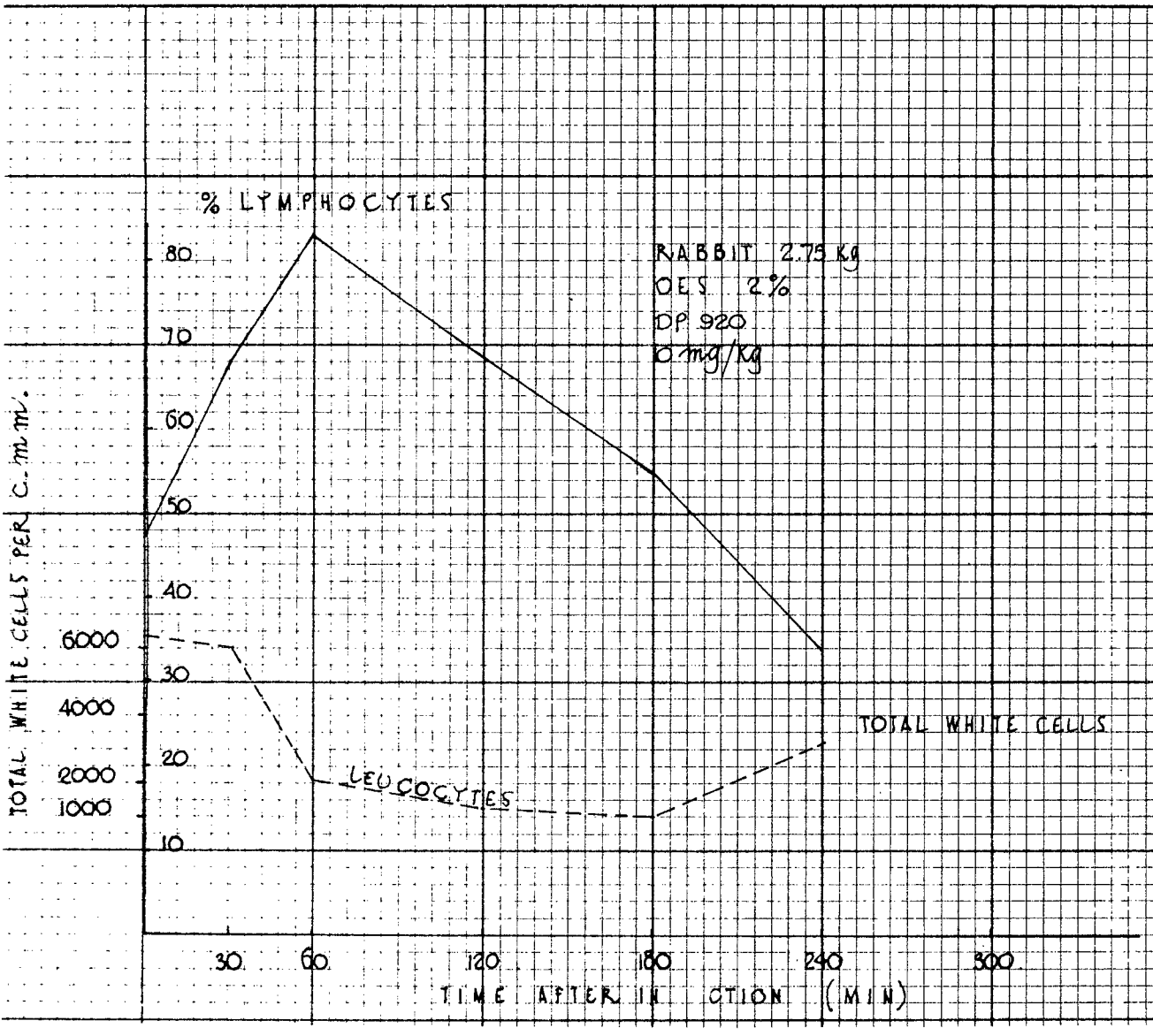


Fig.33

Effect of injection of OES on the total white cell count and on the percentage of lymphocytes

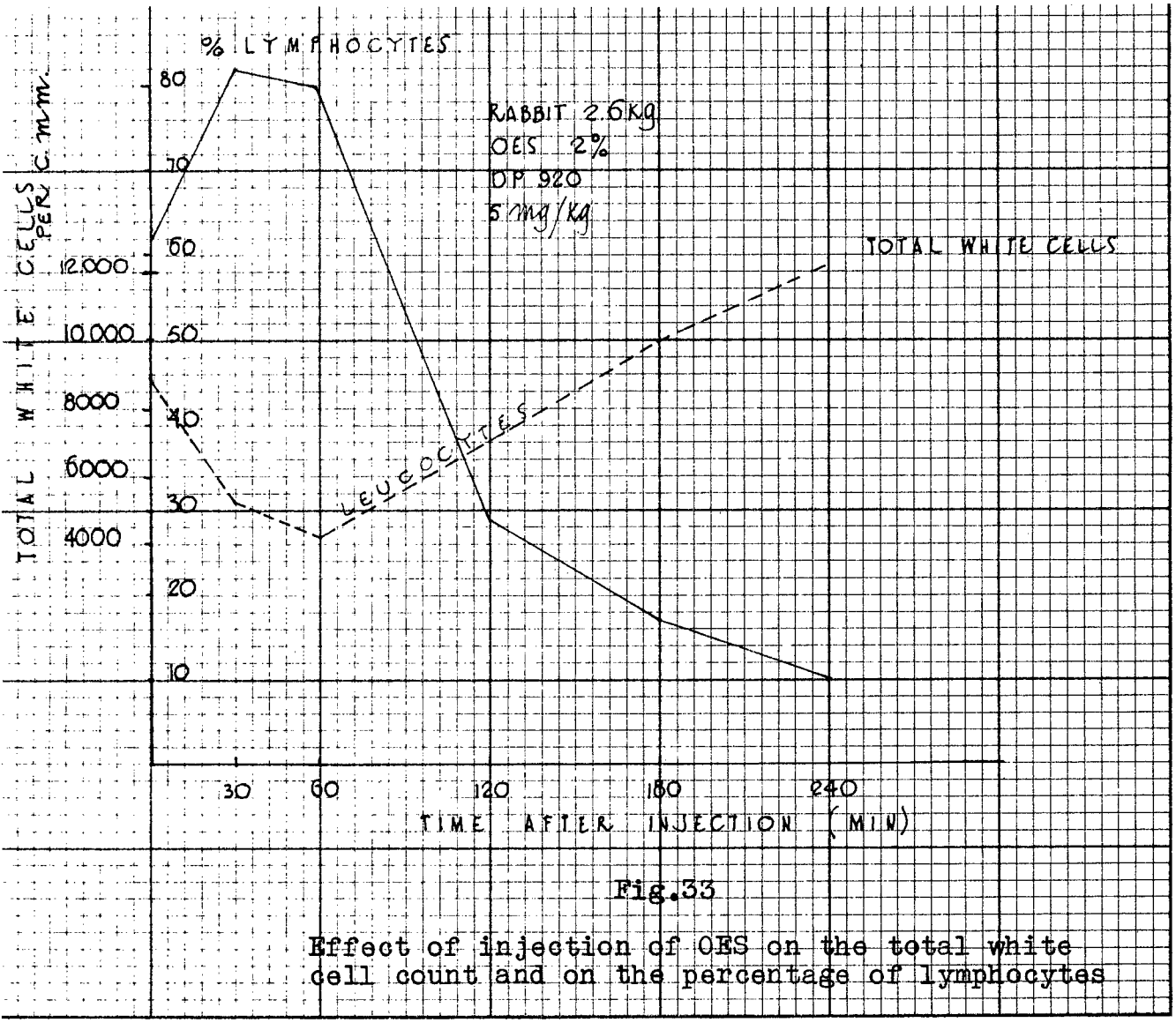


Fig.33

Effect of injection of OES on the total white cell count and on the percentage of lymphocytes

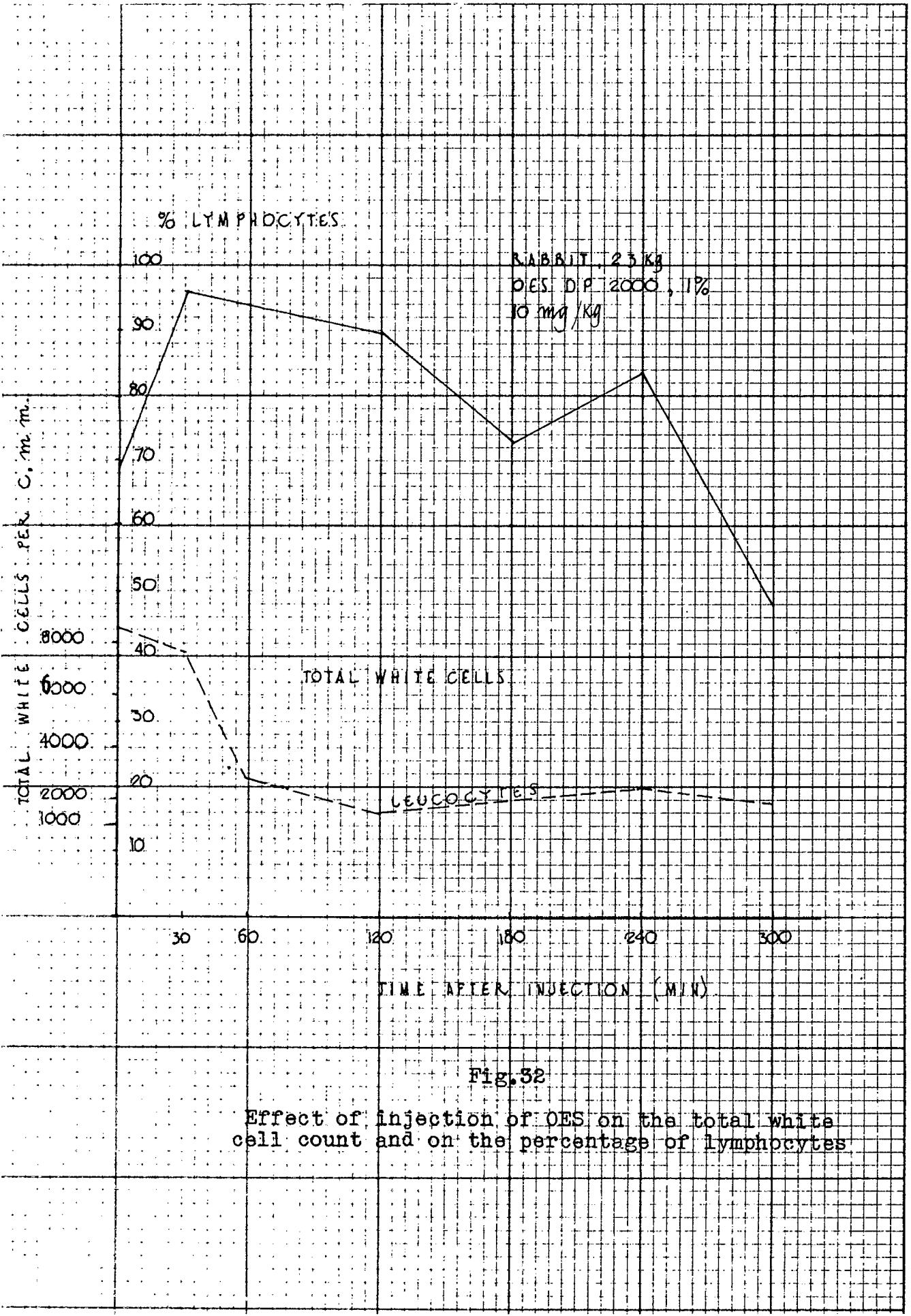


Fig.32

Effect of injection of OES on the total white cell count and on the percentage of lymphocytes

consider that the variations in the count may be explained on a genetic basis.

Spector's Handbook of Biological Data (1956) gives 39 as the lymphocyte percentage in rabbits. These figures are at variance with those obtained by Faludi (1938) and Bushnell and Bangs (1926) who found 50-60% and 56% respectively in rabbits, and are also at variance with those found by the author whose results in the experiments under review vary between 44% and 70%, with a mean of 57.7%. The total white cell count, however, averaged 7,500 which is close to the figures of the other workers mentioned. It is considered unlikely that the genetical variety of the rabbits used can account for the variation in normal blood cell values. The experiments by Faludi were performed in Hungary and those by the author in Germany and South Africa. Figs. 32-35 below indicate the effect of OES on the white blood cell count and Figs. 36 and 37 give the result of control experiments.

The influence of polysaccharide derivatives on the rate of sedimentation (ESR).

Investigation into the action of cellulose derivatives, gelatin and fibrinogen, showed that the ESR increased with an increase in the molecular weight of the substance injected (Thorsen and Hint, 1950; Ryttinger, Swedin and Aberg, 1952; Wiedersheim et al., 1953). As the molecular and chemical structure of the substances investigated varied considerably, it was desirable to use well-defined high-polymer compounds to demonstrate their action on the ESR. The following substances were investigated:

Methylcellulose, with a DP of 12, 50, 160, 360, and 500.

Xylan, with a DP of 100.

Xylan-sulphuric acid ester, with a DP of 12 and 100 and with a degree of substitution (SO_3) of 1% and 18% respectively.

Mannan, with a DP of 400.

Dextran, with a DP of 100 and 1,000.

Glycogen, with a DP of 200 and 8,000.

Methylamylopectin, with a DP of 210.

Oxyethylstarch, with a DP of 170 and 2,000.

Rabbits weighing 2.5 - 3.5kg. were used, the blood being taken from the ear vein 10, 30, 60, 120, 180, 240 and 300 minutes after the injection. The blood samples (0.2ml.) were collected in a paraffined dish and mixed

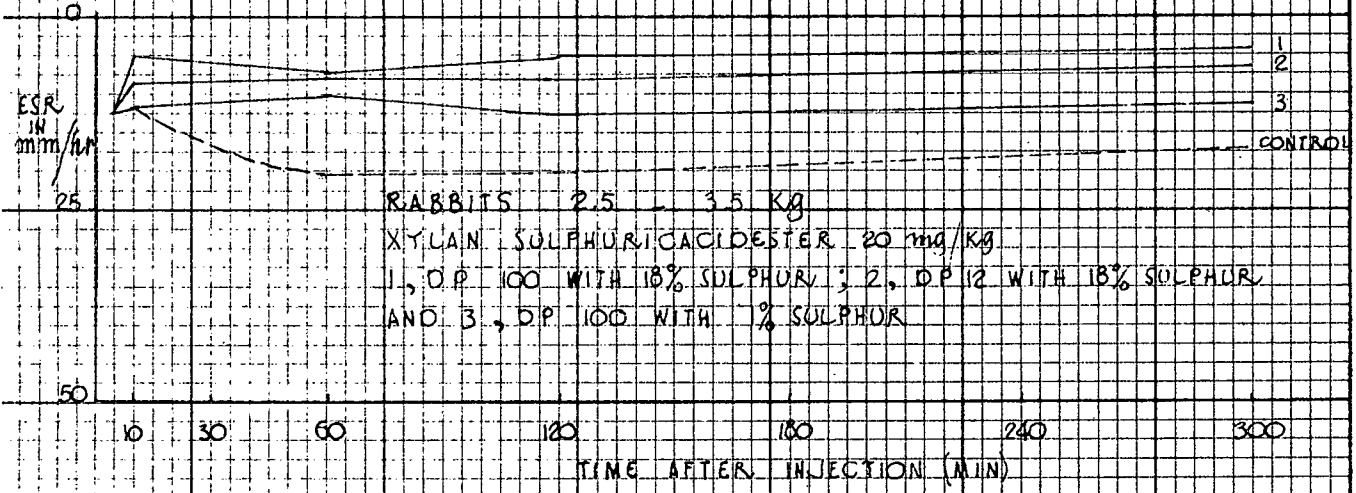


Fig.40

Effect of injection of xylan-sulphuric acid ester of various degrees of substitution (SO_3) on the ESR

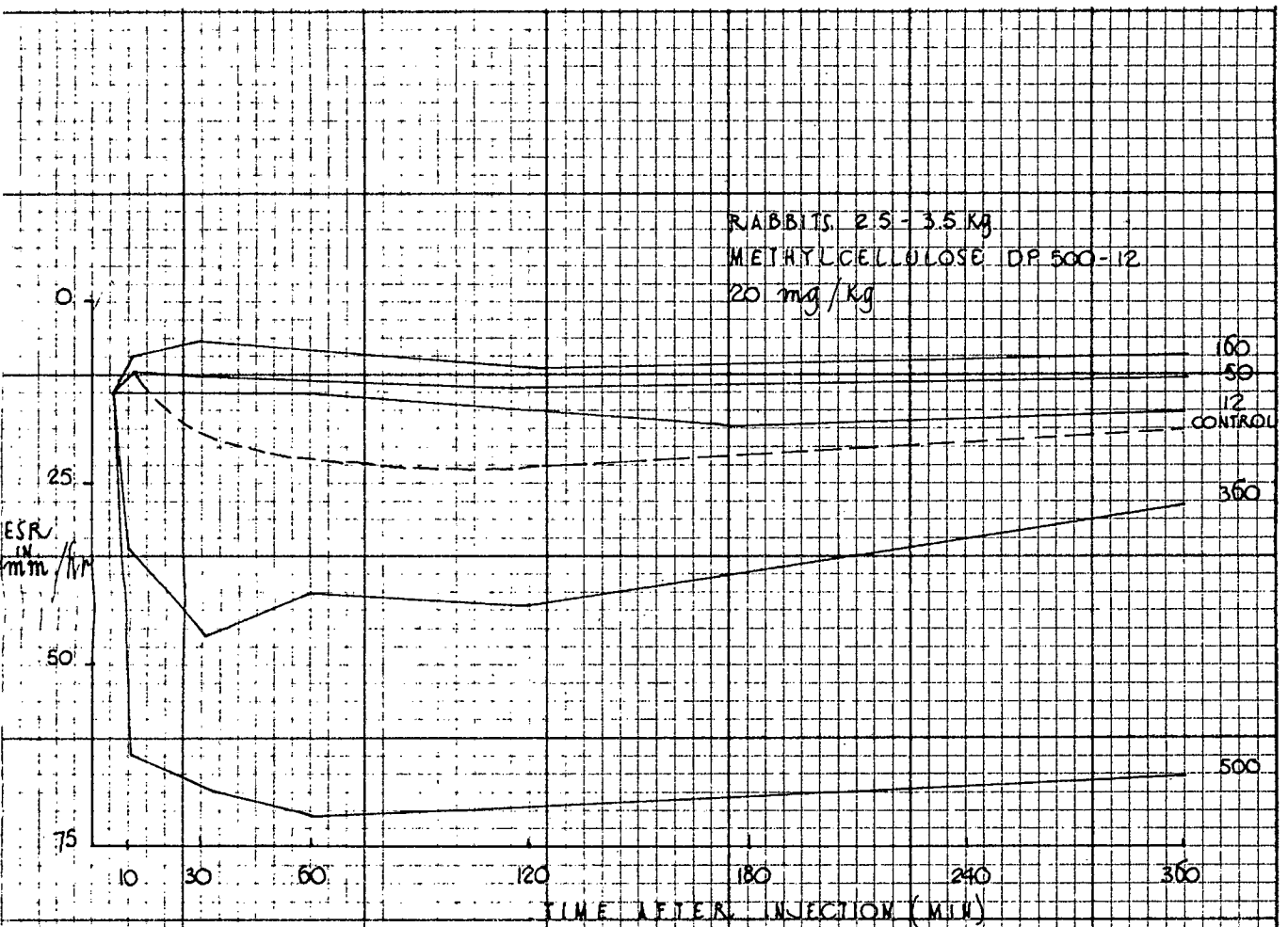


Fig.38

Effect of injection of methylcellulose on the ESR

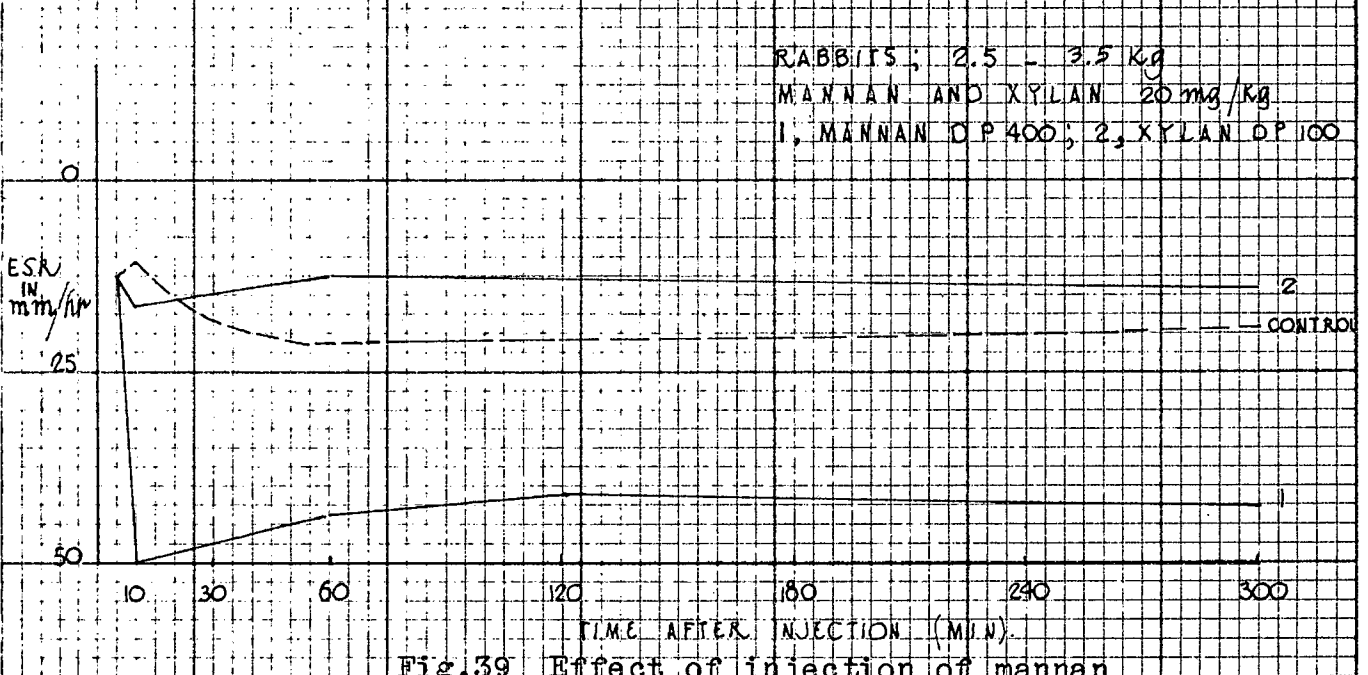


Fig.39 Effect of injection of mannan and of xylan on the ESR

with 0.08ml. of 3.8% sodium citrate. The citrated blood was sucked into micropipettes (Kowarski, 1931) and the sedimentation rate measured. Only rabbits with a normal ESR of 1-3mm./hr. were used and this value taken as the base line. The reading of the ESR values took place 1, 2, 6, and 24 hours after the withdrawal of the blood samples which, in turn, were taken 10, 30, 60, 120, 180, 240, and 360 min. after injection. The 6-hour reading was plotted in all the curves given and the ESR, after injection of 20mg./kg. of methylcellulose in various degrees of polymerisation is shown in Fig. 38. The less the degree of polymerisation, the less pronounced was the sedimentation rate of the red blood cells. Fig. 39 shows xylan and mannan, applied in the same way as methylcellulose, and its influence on the ESR, corresponding to values obtained with methylcellulose of a similar degree of polymerisation. The xylan-sulphuric acid ester, being heteropolar, inhibited the normal ESR to such an extent that, 24 hours after injection, hardly any sedimentation of the red blood cells had occurred. The polarity of the xylan-sulphuric acid ester depends on its degree of SO_3-substitution; variations in this SO_3 content of the molecule had very little influence on the ESR. Fig. 40 shows the ESR after injection of xylan-sulphuric acid ester

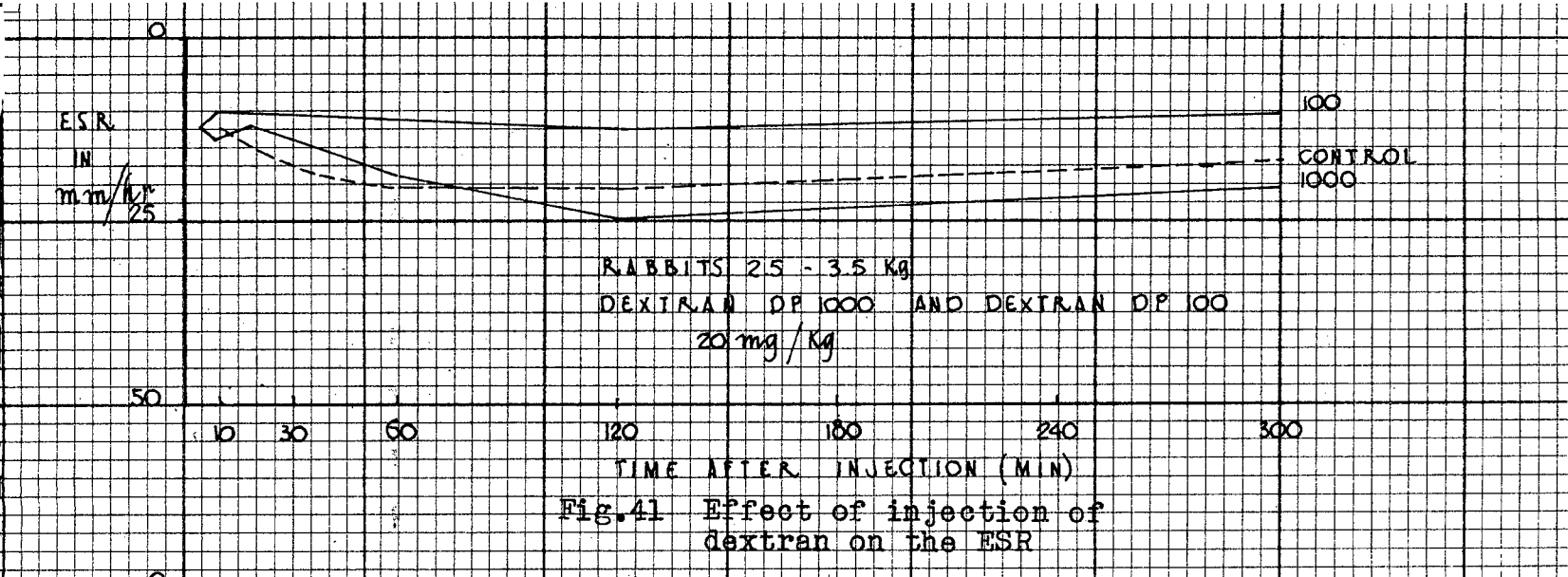


Fig.41 Effect of injection of dextran on the ESR

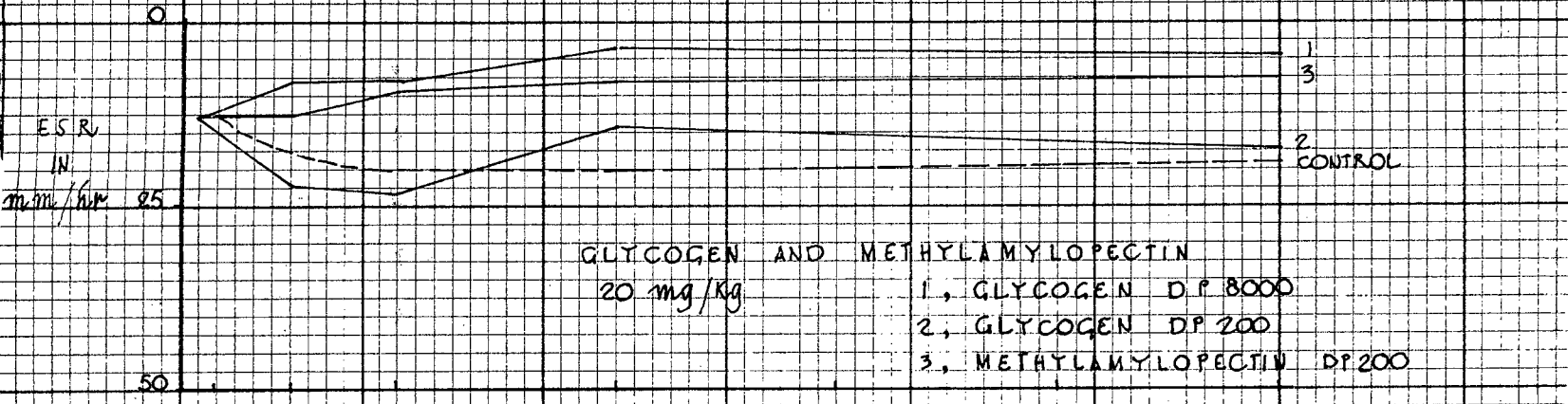


Fig.42 Effect of injection of glycogen and of methylamylopectin on the ESR

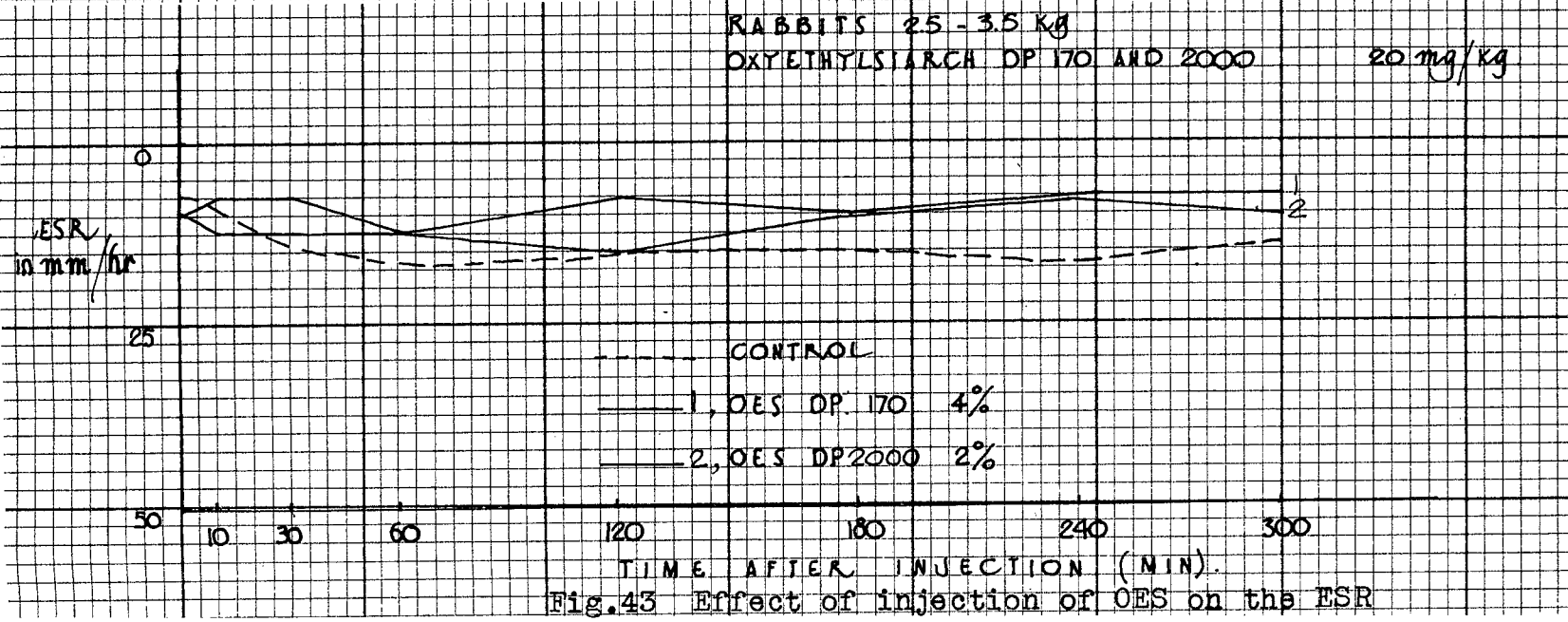


Fig.43 Effect of injection of OES on the ESR

The sedimentation rate of red blood cells either fell or was not affected by injections of substances with branched molecules such as dextran, glycogen, or methylamylopectin. Dextran inhibited the ESR when the degree of polymerisation did not exceed 1,000 thereby confirming the work of Ryttinger et al. (1952), Thorsen and Hint (1950), and Swedin (1936). These authors found that dextran with a degree of polymerisation of 800 or more increased the ESR slightly. Figs. 41 and 42 show the ESR of dextran, glycogen, and methylamylopectin. Oxyethylstarch, injected in the same dose of 20mg./kg. had no effect on the ESR or reduced it slightly, as shown in Fig. 43.

OES AS A BLOOD VOLUME EXPANDER

The search for blood volume expanders increased during the 1939-45 war and produced, amongst other high-polymer compounds of practical importance, polyvinyl-pyrrolidone (Hecht and Weese, 1943), dextran (Grönwall and Ingelmann, 1943) and oxypolygelatin (Campbell, Koepfli, Pauling, Abrahamsen, Dandliker, Feigen, Lanni and Le Rosen, 1950). Blood volume expanders should eventually be eliminated from the circulation or metabolized by body tissues. Polyvinylpyrrolidone, although used successfully during the second world war, is not metabolized and only small amounts are excreted. It remains in the body tissues where it may cause undesirable effects such as tissue irritation, growth of fibrous tissue, or even damage to liver and kidney. Methylcellulose of a high degree of polymerisation keeps the blood pressure of exsanguinated animals high enough to prevent shock; it is nevertheless not metabolized or eliminated from the body tissues and therefore does not fulfil one of the essential requirements of blood volume expanders. The preliminary investigation of OES (Wiedersheim, 1956) showed that most of these criteria are fulfilled, i.e. it is chemically inert, non-toxic, non-allergy forming, and readily excreted. The basic material (starch) is cheap

Table 22

Blood pressure of cats exsanguinated to shock level

Cat No.	Weight	Sex	Blood pressure after exsanguination	Duration of experiment
1.	2.3 kg	male	35 to 50 mm Hg	65 min.
2.	2.0 "	female	35 to 45 " "	125 "
3.	1.8 "	"	35 to 40 " "	90 "
4.	1.7 "	"	40 to 55 " "	120 "
5.	1.9 "	"	35 to 50 " "	170 "
6.	2.0 "	"	35 to 50 " "	75 "
7.	3.5 "	male	45 to 60 " "	45 "
8.	2.9 "	female	50 to 70 " "	140 "

Fig.44

Effect of exsanguination to shock level on the blood pressure of cat no.2

and therefore the production of OES is less expensive than that of dextran. The controlled steps in producing OES guarantee a molecule of fairly uniform size due to the gradual hydrolysis and there is, moreover, no uncertain factor such as bacterial activity, as in the preparation of dextran with Leuconostoc mesenteroides.

In the experiments described cats were used to demonstrate the effect of OES as a blood volume expander. The blood pressure experiments were based on the fact that cats, exsanguinated to a constant blood pressure of 35-45mm.Hg. would die eventually if the blood volume were not replenished. This was demonstrated on 8 cats (Table 22), which died from 1-3 hours after their blood pressure was lowered to between 35 and 70mm.Hg. None of these cats showed any spontaneous rise of the blood pressure after the exsanguination to shock levels. Fig. 44 shows one record of a cat's blood pressure in such an experiment. For the purpose of restoring blood volume OES of a degree of polymerisation of 500 was used. The OES was dissolved in mammalian Ringer as a 4% solution and infused at body temperature into the femoral vein of the animal, which had been anaesthetized with phenobarbitone 30mg./kg. The blood was withdrawn

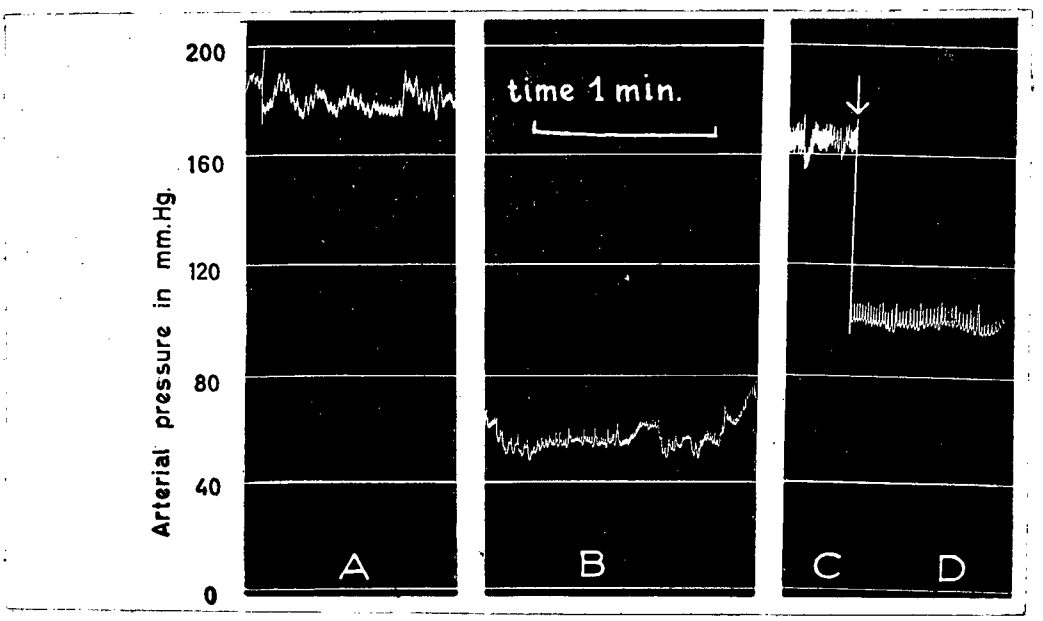


Fig.47

Arterial pressure in cat(3.2 kg). A - before exsanguination; B - after exsanguination; C - 10 min.after infusion of 6% dextran; D - 40 min.after infusion of 6% dextran. At ↓ the kymograph was stopped for 30 min.

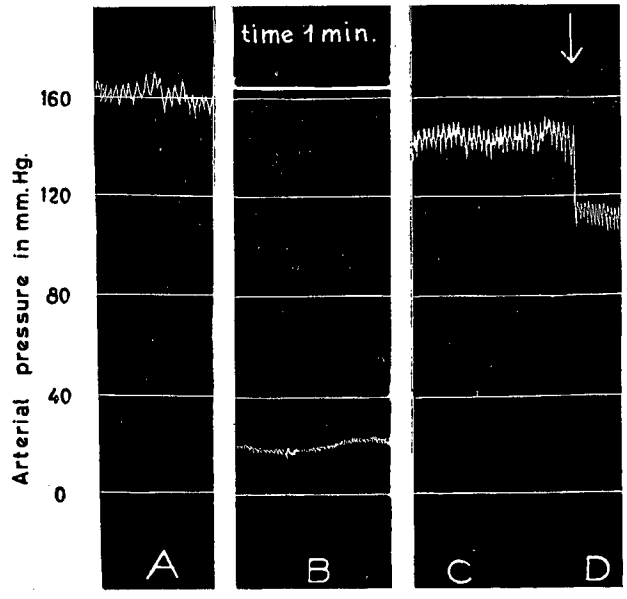


Fig.45 Arterial pressure in cat,(3.4 kg.). A - before exsanguination; B - after exsanguination; C - 10 min. after infusion of 4% OES; D - 40 min.after infusion of 4% OES; At ↓ the kymograph was stopped for 30 min.

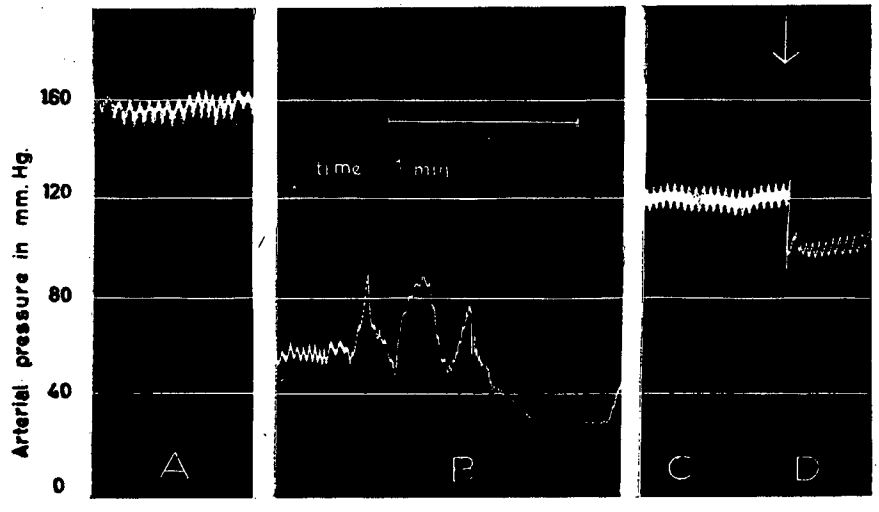


Fig.46 Arterial pressure in cat,(2.8 kg.). A - before exsanguination; B - after exsanguination; C - 30 min.after infusion of 4% OES; D - 60 min.after infusion of 4% OES. At ↓ the kymograph was stopped for 30 min.

Table 23

Blood pressure recordings before and after exsanguination and after infusion.

Experimental animals	Plasma volume expander	B.P. before exsanguin. (mm.Hg.)	B.P. after exsanguin. (mm.Hg.)	B.P. 10 min. after infusion (mm.Hg.)	B.P. 30-40 min. after infusion (mm.Hg.)	B.P. 60-70 min. after infusion (mm.Hg.)	B.P. 90 - 100 min. after infusion (mm.Hg.)
Dog 1	OES (DP 500)	135	55	170	160	150	120
" 2	ditto	180	60	170	170	150	140
Cat 1	ditto	165	60	175	120	120	120
" 2	ditto	160	30	140	110	100	100
" 3	ditto	170	60	130	120	120	90
" 4	ditto	135	30	80	120	120	110
" 5	ditto	130	35	115	125	100	135 (1)
" 6	ditto	160	40	120	120	120	100
" 7	ditto	180	30	120	120	100	80
" 8	ditto	160	60	120	120	100	100
Cat 9	Dextran	150	30	110	110	100	90
" 10	"	180	40	120	110	100	- (2)
" 11	"	130	50	110	110	100	80
" 12	"	180	55	165	100	80	60

(1) Reinfused after 70 min.

(2) Died after 80 min.

from the femoral artery and the blood pressure brought down to between 30 and 50mm.Hg. It was assumed that any spontaneous rise of blood pressure would be seen within 10-20 minutes after bleeding. This being so, blood was again withdrawn until the blood pressure of the animal did not rise any further. Perfusion was then started at a rate of 10ml. per minute until the volume of blood withdrawn had been replaced. Ten minutes after the withdrawal of blood and infusion of OES, the arterial blood pressure did not differ significantly from the blood pressure before exsanguination ($t = 2.13$; $0.1 > P > 0.05$). In every case the blood pressure fell within 1½ hours and the mean blood pressure at the end of this period differed significantly from the pressure before the withdrawal of blood ($t = 2.65$; $0.05 \quad P > 0.02$) (Table 23; Figs. 45 - 47). Dextran, used in the same way as OES, showed similar results. Fig. 47 shows a record of arterial blood pressure in a cat before and after bleeding and infusion with dextran.

Four cats, exsanguinated by the method described above, showed a blood pressure of about 30mm.Hg., indicating a severe collapse of the circulatory system. As a result of this it was not possible to infuse OES from a bottle suspended 30cm. above the cats, and therefore OES was

injected at a higher pressure into the collapsed veins until the blood pressure reached 60mm.Hg. With the same experimental procedure the infusion of dextran similarly failed to maintain the blood pressure in these animals. The fall in blood pressure after dextran infusion was quite marked and indicated that dextran was not superior to OES as a plasma volume expander. As it is known that glomerular capillaries are damaged if the lack of oxygen is severe and prolonged enough (Campbell, 1956) it is not surprising that even high molecular substances pass through the glomeruli. Patients suffering from shock excrete in the urine high-polymer molecules of molecular weight of about 57,000 (Grönwall, Hint, Ingelmann, Wallenius and Wilander, 1952; Wallenius, 1954; Pappenheim, 1955) and dextran molecules of molecular weight as high as 205,000 can pass from the capillaries into the lymph (Grotte, Knudson and Bollman, 1951). It remains to be seen whether OES in its higher molecular fractions shows the same phenomenon and whether its biological actions make it a serious competitor to dextran.

It should however be realized that the uncritical use of high-polymer substances in general, inert as they may appear to be chemically, can cause undesirable alterations in the physiology of blood and of body tissues.

EXCRETION OF OES

The use of OES as a possible plasma volume expander made it desirable to find out more about the duration of the blood plasma/OES concentration after infusion. As the funds to buy tobacco leaf-^{14}C were limited only a few batches of OES-^{14}C were produced: therefore hydrolytically degraded maize starch with high, medium, and low degrees of polymerisation were chosen in order to obtain OES-^{14}C with a DP of approximately 2,000, 900, and 300 respectively.

When the first batch of OES-^{14}C was prepared the viscosity appeared to be much lower than expected. Comparative viscosity measurements of the samples showed that the OES-^{14}C prepared from various batches of degraded starch showed a much lower degree of polymerisation than the non-radioactive batches of OES prepared from the same maize starch. For instance, maize starch degraded for 8 minutes gave a $Z\gamma$ value of 0.2704 and the OES prepared from this gave a $Z\gamma$ of 0.2020, but the OES-^{14}C prepared from the same batch had a $Z\gamma$ of 0.0460. It is likely that this difference in viscosity number was caused by the length of time taken to produce the OES-^{14}C and, perhaps, by the accidental presence of oxygen as polysaccharides in alkaline solutions undergo very

easily auto-oxidative degradation (Staudinger and Jurisch, 1938; Meyer, 1943). This applies to a lesser extent also to starch fraction number 4 (see Table 4), which was degraded for 7 hours. Other fractions were produced from starch fraction numbers 2 and 3 and treated in the complete absence of oxygen and at low temperatures of +4 to +8°C. The OES-^{14}C produced showed much less degradation when its DP was measured compared with that of the raw material which shows very little auto-oxidative tendency (see Table 4).

The OES-^{14}C was tested on 5 dogs to find the disappearance curve of ^{14}C in the plasma which would indicate the rate of excretion of OES, which depends on its molecular weight. The animals were anaesthetized with nembutal and in the first experiment 10ml. of OES with a DP of 460 in 3% solution was injected intravenously. Blood samples were taken from the femoral artery, starting 1 minute after injection and continuing at regular intervals for various periods. The 5ml. blood samples were centrifuged and the plasma kept in a refrigerator. Two days after the experiment was completed the beta-ray emission of the plasma was measured. As the plasma sample dried on an aluminium tray, its surface was found to warp and to break up. In order to prevent this, filter paper was first placed on the tray and, either

Table 26

Dog, 8.5 kg.
β-ray emission of plasma after injection of 20 ml. OES-^{14}C 3% (DP 460)

Sample number	Time blood taken in min. after injection	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	1	0.030	5	334	119	97	3,233
2	1	0.032	11	1316	119	97	3,031
3	2	0.042	8	635	79	57	1,357
4	2	0.039	5	598	67	45	1,153
5	3	0.010	9	577	63	41	4,100
6	5	0.039	6	420	70	48	1,230
7	7	0.032	2	171	85	73	2,281
8	7	0.033	5	333	66	44	1,333
9	10	0.026	8	488	61	39	1,500
10	10	0.024	6	358	59	37	1,541
11	15	0.026	5	292	58	36	1,384
12	20	0.025	5	270	54	32	1,280
13	20	0.026	26	1165	45	23	884
14	30	0.024	5	172	34	12	500
15	30	0.023	5	181	36	14	608
16	45	0.023	34	1119	33	11	478
17	45	0.024	5	161	32	10	416
18	60	0.023	6	188	32	10	434
19	60	0.029	4	124	31	9	310
20	90	0.024	5	161	32	10	416
21	90	0.022	5	150	30	8	363
22	120	0.023	8	215	27	7	305

Background count: 22 c.p.m.

Table 24

Dog, 9.0 kg.
β-ray emission of plasma after injection of 10 ml. OES-^{14}C 3% (DP 460)

Sample number	Time blood taken in min. after injection	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	1	0.068	5	278	56	32	470
2	5	0.139	5	355	71	47	338
3	7	0.071	5	167	33	9	127
4	10	0.140	5	166	33	9	64
5	20	0.136	5	151	30	6	44
6	60	0.146	5	146	29	5	34
7	90	0.072	5	135	27	-	-
8	120	0.068	5	137	27	-	-
9	180	0.072	5	129	26	-	-
10	260	0.149	5	122	24	-	-

Background count: 24 c.p.m.

0.3 ml. plasma, glucose filter paper method - used in all experiments including Table 30.

Table 25

Dog, 9.0 kg.
β-ray emission of urine after injection of 10 ml. OES-^{14}C 3% (DP 460)

Sample number	time urine taken in min. after injection	weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	15	0.002	5	311	62	38	19,000
2	30	0.002	5	324	65	41	20,500
3	60	0.014	5	2596	519	495	35,357
4	90	0.008	5	1562	312	288	36,000
5	120	0.016	5	1717	343	319	19,937
6	150	0.020	5	1426	285	261	13,050
7	180	0.039	5	1961	392	368	9,436
8	240	0.046	5	1657	331	307	6,674
9	300	0.023	5	2463	483	461	20,043

Background count: 24 c.p.m.

treated with a 10% glucose solution, or glued onto the tray with an adhesive dissolved in acetone. It is essential that warping of filter paper and plasma be prevented since the distance from the end-window of the counter must be constant for all the samples counted. It was found that a sample at 2.0cm. from the end-window gave 8,039 counts per minute (c.p.m.); the same sample placed 1.0cm. nearer to the end-window gave 10,582 c.p.m., a difference of 2,543 counts per minute.

The first experiment was carried out on a female dog of 9kg. body weight. 10ml. OES of DP 460 in 3% solution was injected intravenously and the β -ray emission of the plasma was counted (Table 24). It showed low activity. Urine, collected with a catheter, showed a very high activity of β -ray emission (Table 25).

As the activity of the plasma obtained in Table 24 was low the amount of OES injected was increased in all following experiments and aliquots of 0.3ml. of plasma were put onto each tray. A male dog of 8.5kg. body weight was injected with 20ml. of a 3% solution of OES; the results, given in Table 26, showed a higher activity. This dog died of air embolism due to a leak in the infusion pump. Urine samples from the same animal taken 35, 80, and 120 minutes after the injection of OES-^{14}C showed a strong activity at first but this decreased

Table 27

Dog, 8.5 kg.
β -ray emission of urine after injection of 20 ml. 3% OES-^{14}C (DP 460)

Sample number	time urine taken in min. after injection	weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	35	0.015	4	6912	1728	1742	11,631
2	80	0.014	1	973	973	960	6,857
3	120	0.011	2	1067	533	513	4,663

Background count: 22 c.p.m.

Table 28

Dog, 7.5 kg.
β-ray emission of plasma after injection of 20 ml. 3% OES-^{14}C (DP 900)

Sample number	Time blood taken in min. after injection	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	1	0.016	5	907	181	157	9,812
2	2	0.017	5	708	156	132	7,764
3	3	0.018	5	711	142	118	6,555
4	5	0.018	5	576	115	91	5,055
5	7	0.018	5	536	107	83	4,611
6	10	0.018	5	449	90	66	3,666
7	15	0.017	5	396	79	55	3,235
8	20	0.018	5	344	69	45	2,500
9	30	0.018	5	341	68	44	2,444
10	45	0.018	5	251	50	26	1,444
11	60	0.018	5	214	43	19	1,055
12	90	0.019	11	573	52	28	1,473
13	120	0.018	5	223	45	21	1,166
14	180	0.020	5	177	35	11	550
15	240	0.019	4	180	65	41	2,157

Background count: 24 c.p.m.

later on (Table 27).

This experiment was repeated with a higher molecular fraction (DP 900) and blood plasma samples were taken and counted in the same way as before. The results in this experiment differed very little from the results in Table 26 in which an OES with a DP of 460 was used. The higher c.p.m./g. was caused by the greater activity of the labelled OES but the rate of excretion was approximately the same. In this experiment 20ml. of a 3% solution of OES-^{14}C with a DP of 900 injected intravenously into a male dog of 7.5kg., and the results are shown in Table 28. The decrease of plasma ^{14}C activity in both cases (Tables 26 and 28) showed that the difference in molecular size did not appreciably influence the rate of excretion or the deposition of OES. This may, however, not be the case when OES-^{14}C with a DP of 2,000 is used in future experiments.

Experiments in nephrectomised dogs

In order to investigate whether the decreasing activity of ^{14}C in plasma is caused by excretion or by the storage of OES in various tissues, 35ml. of a 3% solution of OES (DP 460) was injected intravenously into a dog which had been bilaterally nephrectomised. As before, blood samples taken from the femoral artery were

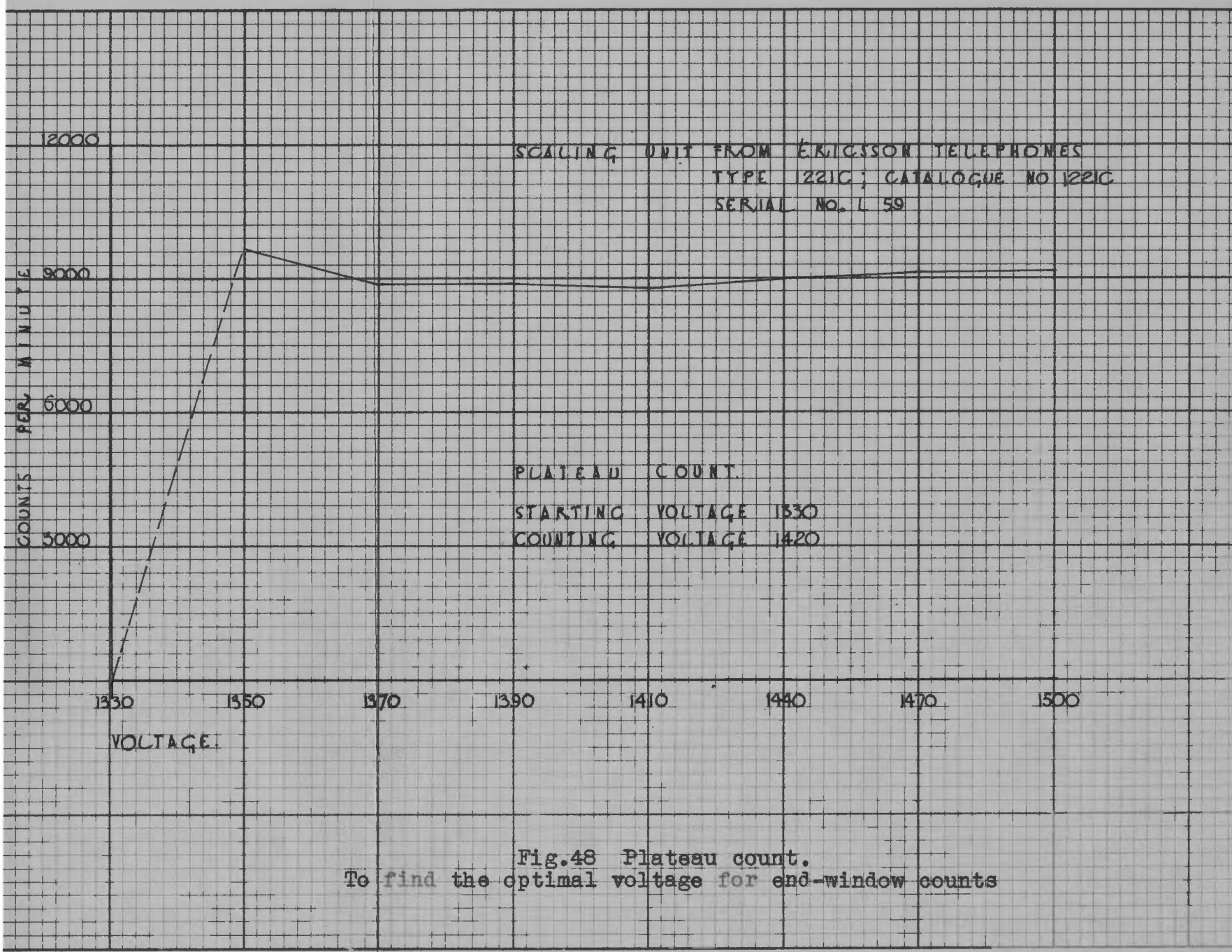


Fig.48 Plateau count.
To find the optimal voltage for end-window counts

Table 30

Nephrectomized dog, 9.9 kg.
β-ray emission of plasma after injection of 100 ml. 3% OES-^{14}C (DP 900)

Sample number	Time blood taken in min. after injection	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	1	0.0358	10	5346	536	521	14,472
2	1	0.0335	5	2837	567	553	16,757
3	2	0.0345	2	2028	507	491	14,441
4	2	0.0344	2	1004	502	586	14,294
5	3	0.0347	1	558	558	536	15,314
6	3	0.0143	7	2247	321	305	21,786
7	5	0.0392	5	2059	412	396	10,153
8	5	0.0525	5	2415	483	467	8,980
9	7	0.0321	8	4051	506	490	15,312
10	7	0.0367	1	552	552	536	14,604
11	10	0.0344	2	1004	502	486	14,294
12	10	0.0355	3	1675	558	536	15,314
13	15	0.0460	2	972	486	470	10,217
14	15	0.0337	2	885	443	427	12,558
15	20	0.0352	3	1354	451	535	12,428
16	20	0.0362	4	1886	472	456	12,667
17	30	0.0344	2	851	426	410	12,058
18	30	0.0390	6	2408	401	385	9,871
19	45	0.0323	1	428	428	412	12,875
20	45	0.0371	1	431	431	415	11,216
21	60	0.0334	2	706	353	337	10,212
22	60	0.0334	2	852	426	410	12,424
23	90	0.0434	2	742	371	355	8,255
24	90	0.0367	4	1464	366	350	9,459
25	120	0.0380	2	731	366	350	9,210
26	120	0.0292	2	794	397	381	13,137
27	165	0.0299	4	1271	319	303	10,100
28	165	0.0341	1	253	253	237	6,970
29	Urine 165	0.0491	3	32	11	-	-
30	Bile 165	0.1330	3	230	77	61	-

Background count: 16 c.p.m.

Table 29

Nephrectomized dog, 9.6 kg. Nembutal anaesthesia.
β-ray emission of plasma after injection of 35 ml. 3% OES-^{14}C (DP 460)

Sample number	Time blood taken in min. after injection	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
1	1	0.048	6	1641	273	249	5,187
2	2	0.043	9	1982	220	196	4,558
3	5	0.046	13	2671	205	181	3,934
4	8	0.047	7	1334	190	166	3,531
5	10	0.047	5	858	172	148	3,148
6	15	0.046	8	1277	159	135	2,934
7	20	0.066	4	660	165	141	2,136
8	30	0.046	5	613	123	99	2,152
9	45	0.047	5	551	110	86	1,829
10	60	0.047	4	400	100	76	1,617
11	90	0.047	7	716	102	78	1,659
12	120	0.045	7	643	92	68	1,511
13	180	0.047	5	306	61	37	787
14	240	0.046	6	425	71	47	1,021
15	360	0.031	4	255	63	39	1,258
16	420	0.031	5	284	57	33	1,064

Background count: 24 c.p.m.

centrifuged and the plasma measured. Table 29 shows that the radioactive OES disappeared from the blood stream at almost the same rate as in the previous experiments (Tables 24, 26 and 28).

In a subsequent experiment 100ml. of OES-^{14}C with a DP of 900 was injected intravenously into a nephrectomized dog of body weight 9.9kg. (Table 30). For this experiment an Ericsson scaling unit (Type 1221C, Cat.No. 1221C, Serial No. L59) was used together with a lead castle end-window, as before. In this experiment the plasma activity decreased less markedly than in the experiment shown in Table 29.

To check the reliability of the Geiger-Müller tube and of the scaling unit, a plateau count was taken and the β- emissions between the range of the starting voltage (1330V.) and the counting voltage (1420V.) were measured. Fig. 48 shows the graph of such a plateau count, which is well within the limits allowed for experimental error.

DISTRIBUTION OF OES IN THE BODY

The distribution of OES-^{14}C in various organs of a nephrectomized dog was investigated, the organs being excised and prepared for end-window counts. As the samples of the various powdered organs could not be comparably spread on the sampling tray (for example, the pulverized liver was much finer than the pulverized lung tissue), the method adopted for estimating radioactivity in plasma and in urine had to be rejected as unsuitable for solid organs and another method had to be found.

The organs under investigation were digested with 2N-NaOH, which seemed to be an adequate method of achieving a uniform solution and a smooth surface. The dissolved tissue, however, being strongly alkaline, caused a chemical reaction between the NaOH and the aluminium tray, resulting in a slow but steady rise of foamy matter; there was a gradual increase β-ray emission of the sample and a danger of contamination of the mica end-window. This method had, therefore, to be abandoned.

An attempt was then made to neutralize the NaOH tissue solution but this caused precipitation of organic matter previously dissolved, resulting in an uneven surface when dry. The trays were coated with paraffin-

Table 31

Nephrectomized dog, 9.9 kg.
β-ray emission of various tissues after injection of 100 ml. 3% OES-^{14}C (DP.900)

Organ	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g.
Lymph	0.2700	5	138	28	5	18
"	0.3694	5	146	29	6	16
Spleen	0.3716	12	297	25	2	5
"	0.3670	5	136	27	4	11
Kidney	0.3262	4	97	24	1	3
"	0.3620	5	129	26	3	8
Liver	0.3688	9	230	25	2	5
"	0.4056	7	201	26	3	7
Dia-phragm	0.4155	5	130	26	3	7
	0.4161	4	109	27	4	9
Fat	0.3398	8	192	24	1	3
"	0.3582	7	176	25	2	5
Lung	0.3407	13	360	27	4	12
"	0.3681	15	400	27	4	11

Ericsson Scaling Unit, Serial No. L 62.

Background count: 23 c.p.m.

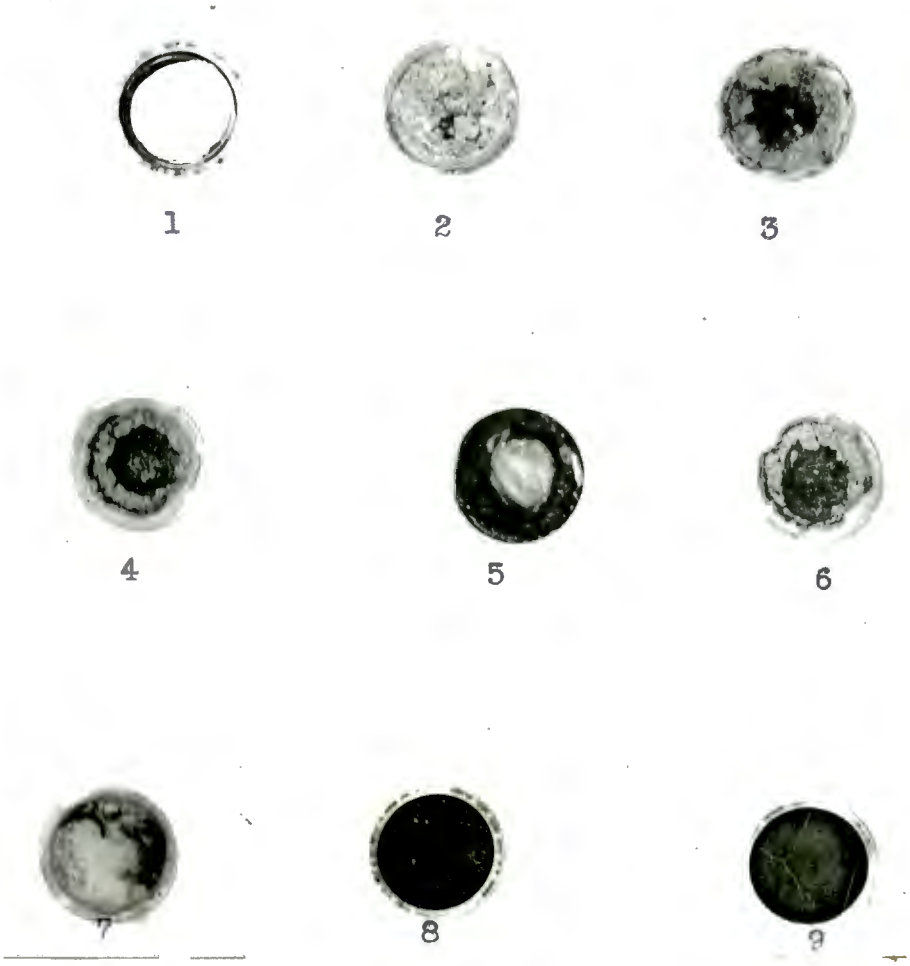


Fig.49 Comparison of the effectiveness of various protective coatings in preventing reaction between the NaOH solution and the aluminium tray.

Trays 1-2 untreated
1,empty
2,full

Trays 3-9 treated with:
3,paraffin wax
4,carnauba wax
5,Pliobond solution
6,silicone solution
7,Nobecutane(sprayed once)
8,Nobecutane(sprayed twice)
9,Perspex-chloroform solution

wax, but this covering gave insufficient protection. Carnauba wax was then tried and gave a very firm coating but showed little improvement on paraffin-wax. Synthetic rubber proved to be similarly useless. Finally, trials with sprays of Nobecutane, a plastic dressing for surgical use, gave satisfactory results as did Perspex dissolved in chloroform into which the trays were dipped. Photographs of trays under various conditions are given in Fig. 49.

The organs whose β-ray emissions were estimated, were dried for 24 hours at 38°C and small portions were weighed and dissolved in 2N-NaOH. The final concentration for all organs was 1g./4ml. of 2N-NaOH (25% solution) of which 0.5ml. was put onto the plastic coated tray and dried. Weighing of samples and coating of trays were carried out as previously described. Table 31 shows the β-ray emission of the excised organs. From this table it is clear that the activity of OES-^{14}C was not strong enough in the different organs to show clear results when counted. This was due either to the self-absorption of ^{14}C in the samples, or to the small quantities of ^{14}C injected. It was not possible at that time to obtain a ^{14}C source with a higher specific activity and attempts were made to obtain results by injecting greater volumes and higher concentrations of OES.^{14}C.

Table 33

Rats, 200-415 g.

β-ray emission of various tissues prepared for autoradiography after injection of OES-^{14}C, 3%, (DP 190, 460 and 900).

Rat No.	Weight in g.	3% OES-^{14}C injected DP.	ml.	Survival time after injection
1	200	190	5.5	1 hour
2	270	190	7.0	6 hours
3	320	190	8.0	1 day
4	415	190	10.0	3 days
5	350	460	10.0	1 hour
6	280	460	7.5	6 hours
7	265	460	6.0	1 day
8	260	460	6.5	3 days
9	360	900	10.0	1 hour
10	260	900	6.5	6 hours
11	250	900	6.0	1 day
12	260	900	8.0	3 days

A rabbit (2.7kg.) received 30ml. OES-^{14}C (DP 900) in a 4% solution intravenously and was killed 4 hours after the injection. None of the organs investigated gave β-ray emission strong enough to be significantly different from the background count. The very slight increase in radioactivity of kidney and spleen was not considered to give reliable information of the storage of OES.

Another rabbit (3.8kg.) received 80ml. of a 5% solution of OES-^{14}C with a DP of 870; four hours after injection the animal was killed and the organs were excised and prepared for the end-window counts. Table 32 gives the results which again showed very little activity.

Storage of OES-^{14}C in the tissues (autoradiography)

Anaesthetized male rats were injected intravenously (thyroid vein) with various fractions of OES-^{14}C and killed from 1 hour to 3 days later. (Table 33). Samples of the liver, spleen, heart, lung, and kidney were prepared histologically as follows:

The tissues were fixed in Bouin's fixative, embedded in the usual manner, and cut into the thinnest possible sections (7-5μ). A wet mounting technique was applied in which the tissue sections float on warm water to

Table 32

Rabbit, 3.8 kg.
β-ray emission of various tissues after injection of 80 ml. 5% OES-^{14}C (DP 870).

Organ	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g
Kidney	0.4734	25	614	28	9	19
Heart	0.5683	5	159	32	13	23
Lung	0.4121	10	120	12	-	-
Liver	0.4890	10	211	21	2	4
Spleen	0.6264	10	82	8	-	-
Plasma	0.0835	20	863	43	24	289

Background: 19 c.p.m.

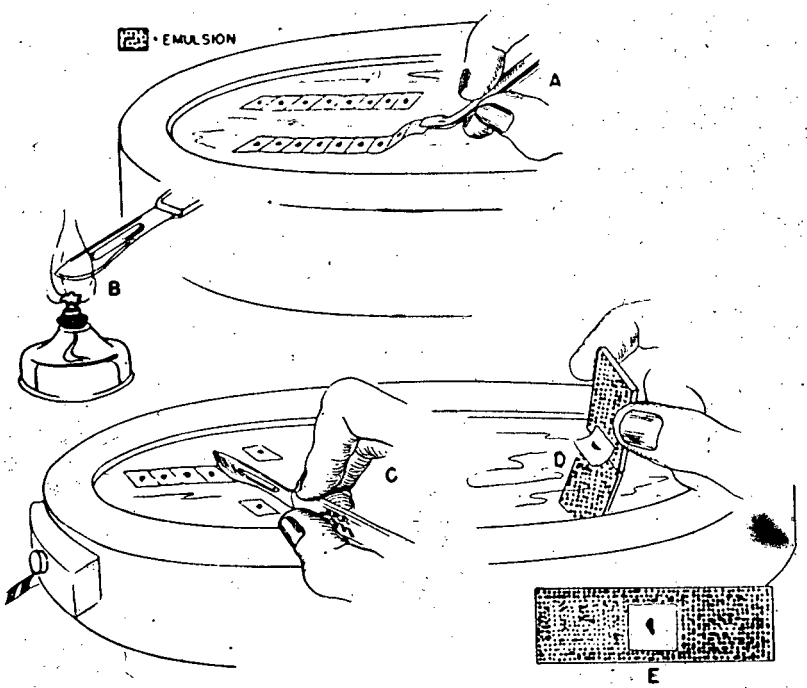


Fig.50

Technique of mounting tissue section,using the wet method

remove wrinkles. With the heated blade of a knife one or more sections were separated from the ribbon and scooped up by placing the NTB plate below them in the water in order to obtain a permanent mounted section on the emulsion (Fig. 50). The plates were carefully dried under a stream of warm air, pressed together very tightly, sealed in a light-proof box, and stored in a refrigerator.

Although the exposure time for weak β -rays can be 6 months or more (Boyd, Casarett, Altmann, Noonan and Salomon, 1948; Skipper, Nolan and Simpson, 1951; Boyd, 1955) the first batch, using Eastman D19 developer and 3% hypo as a fixative, was developed 4 months later. All the films were blank. A further 2-4 months exposure time seem to be advisable before the final deposition of OES can be traced. On the other hand, it is possible that the OES-^{14}C used was too weak to give results.

Investigation into storage of radioactive glycogen

It was of interest to find out whether the OES-^{14}C could have been metabolised and detected as glycogen-^{14}C. A rabbit weighing 2.6kg. was injected intravenously with 6ml. of 1.5% solution of OES-^{14}C (DP 360) at 9 a.m., 5 p.m., and again, the following day at 8 a.m. and was

killed 3 hours thereafter. The liver (63g.) was excised and the glycogen estimated, using the method of Stetten, Katzen and Stetten (1956) and Stetten, Katzen and Stetten (1958), which was modified by Zwarenstein and van der Schyff (1959) and reads as follows:

The liver was homogenized with 10% trichloracetic acid (TCA) using 5ml. TCA to 3g. liver, and centrifuged for 5 minutes. The residues were again extracted with 3ml. 5% TCA, centrifuged, and 1.2 vol. of 95% ethanol were added to the decanted supernatant fluid, mixed, and kept for 3 min. The mixture was centrifuged, the precipitate dissolved in twice distilled water, and 95% ethanol added to the dissolved precipitate in a ratio of 6 parts ethanol to 5 parts of the glycogen in solution. The glycogen was precipitated again and the solution centrifuged for 3 min. This stage of the production of glycogen was repeated 5 times. The supernatant fluid turned from clear to milky, indicating that the electrolytes had disappeared from the glycogen in solution and thus prevented the latter from being fully precipitated. As this means a gradual loss of glycogen one drop of lithium bromide (saturated solution of lithium bromide in 95% ethanol) was added and the full amount of glycogen precipitated. Nothing remained

Table 34

Rabbit, 2.6 kg.

β-ray emission of liver glycogen after injection of 18 ml. 1.5% OES-^{14}C (DP 870).

No.	Sample	Weight of sample in g.	Time min.	Total counts	C.p.m.	C.p.m. corrected	C.p.m./g
1	Glycogen	0.0586	5	35	7	-	-
2	"	0.0523	10	72	7	-	-
3	TCA	0.0975	5	36	7	-	-
4	Alcohol	0.0037	3	33	11	4	1,333
5	Alcohol	0.0188	3	24	8	-	-
6	Alcohol	0.0011	7	50	7	-	-
7	Alcohol	0.0011	10	68	7	-	-

Background: 7 c.p.m.

in solution and was thus not lost by decantation. Finally, the glycogen was washed twice in each of 52% and 95% ethanol, twice in absolute ethanol, and twice in ether, and was then put into a vacuum desiccator together with phosphorus pentoxide and dried for 15 minutes. The glycogen and the different washings were put onto the counting trays and the β-ray emission counted in the usual way (Table 34). No radioactive glycogen was detected. Judging from previous experiments (Tables 31 and 32) this may have been due to self-absorption of ^{14}C in the samples, or to the fact that OES was not metabolized to liver glycogen.

TOXIN BINDING CAPACITY OF OES

As already stated, high-polymer substances have been used to augment the therapeutic effect of drugs (Pedersen et al., 1950) and the possibility of linking bacterial toxins to macromolecules has been tested in different laboratories (Schubert, 1949, 1950; Hummel et al., 1952). The prophylactic and curative effect of high polymers such as polyvinylpyrollidone (PVP) in cases of diphtheria, tetanus, and botulism intoxication were described by Schubert (1948), Krech (1952), and Dieckhoff and Ludwig (1950). The detoxicating action of PVP was first thought to be due to the ease with which it was excreted, thus allowing the attached toxin to pass through the glomeruli. It was, however, soon established in these experiments that, although the toxin often appeared in the urine together with the PVP, it was sometimes not found there; in these cases it was thought that the toxophoric group of the toxin was combined with the PVP. Whatever view may have been held it is certain that a complex is formed between the toxin and the macromolecule.

In the present investigation OES and methylcellulose with a degree of polymerisation of 920 and 400 respectively were used to test their toxin-binding

properties. Throughout the experiments mice weighing 20-25g. were used. The tetanus toxin was obtained from the Virus Research Unit, Cape Town. Trevan (1927) and Munch and Garlough (1935), and many other workers defined and estimated the lethal dose and on the basis of these investigations the LD_{100} was estimated. Unlike the LD of most drugs, the LD of tetanus toxin can be estimated for a particular day only. For example, the LD for the 3rd day is stronger than that for the 8th day, and so on until a point is reached where all the animals show some signs of tetanus intoxication but none die.

The LD_{100} for the 5th day was $1\mu g$. tetanus toxin per mouse in 0.2ml. Istrati solution (Istrati, 1938). This solution was used to prevent the rapid weakening of the toxin which occurs when the latter is diluted and kept in 0.9%NaCl. Istrati solution consists of 4.75g. $Na_2HPO_4 \cdot 2H_2O$ in 400ml. distilled water plus 1.25g. peptone, previously dissolved in 1.25ml. distilled water. Toxin, having a LD_{100} for the 5th day was used throughout the experiments but was diluted with an equal volume of 0.9%NaCl directly before injection. This dilution was necessary as it had been found that too severe an intoxication obscured any signs of protective action by high polymers injected with the toxin; in

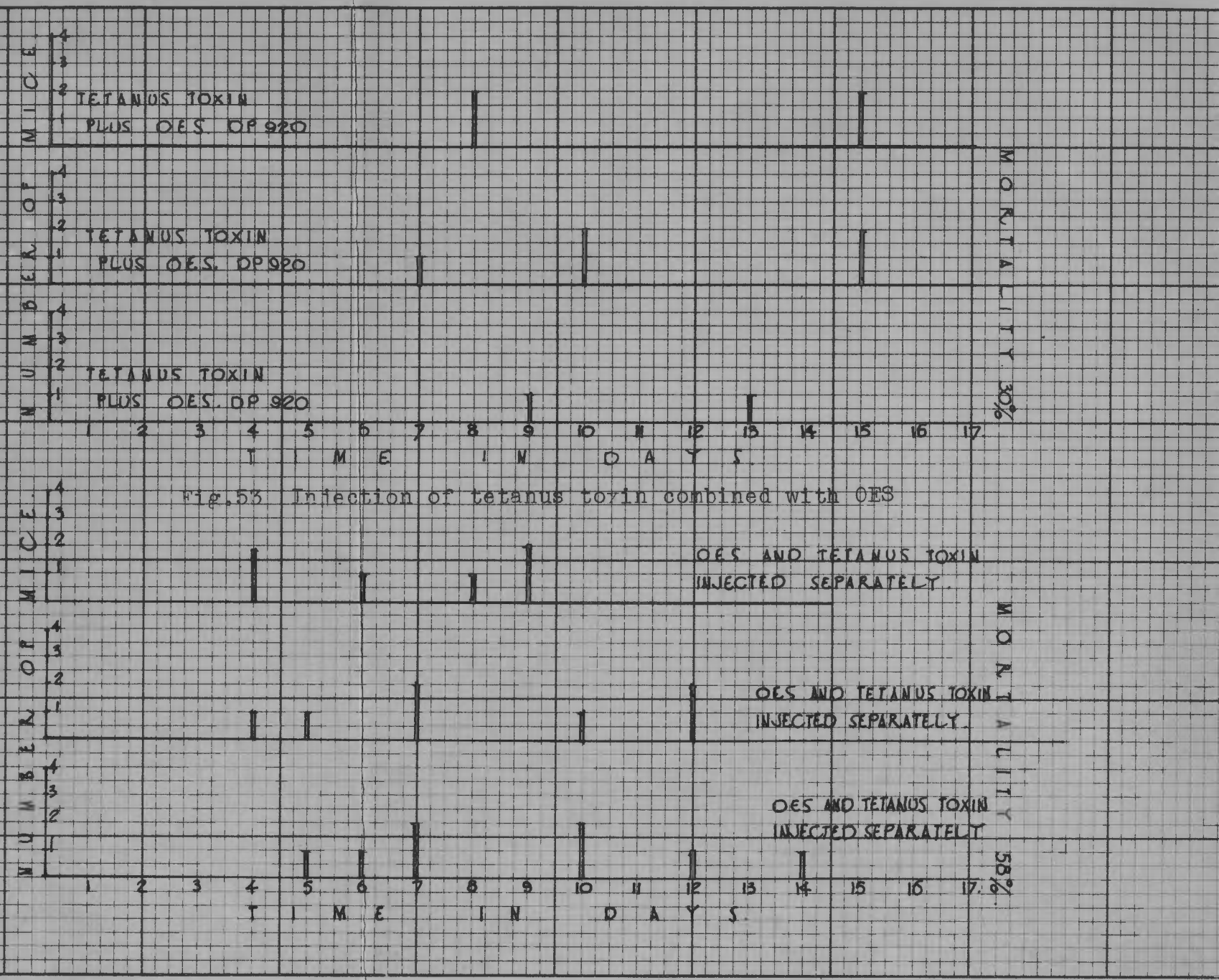
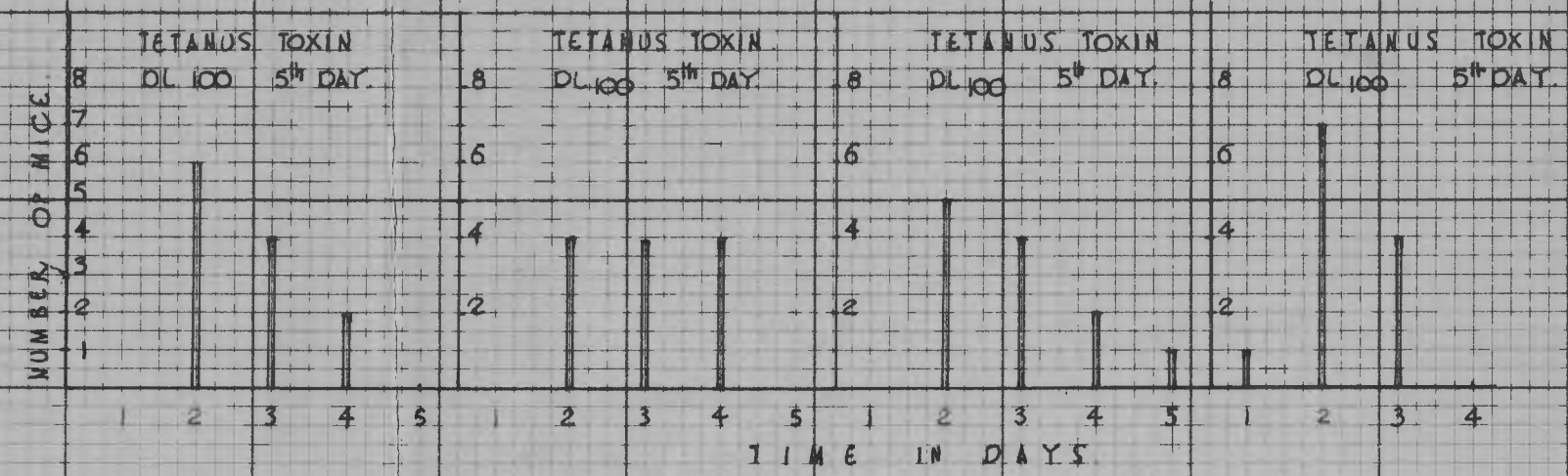


Fig. 54 Effect of tetanus toxin and of OES injected separately



‖ INDICATES DEATH
DL represents lethal dose(LD)

Fig.51 Injection of tetanus toxin to estimate the LD_{100} for the 5th day(40.0 µg./kg.)

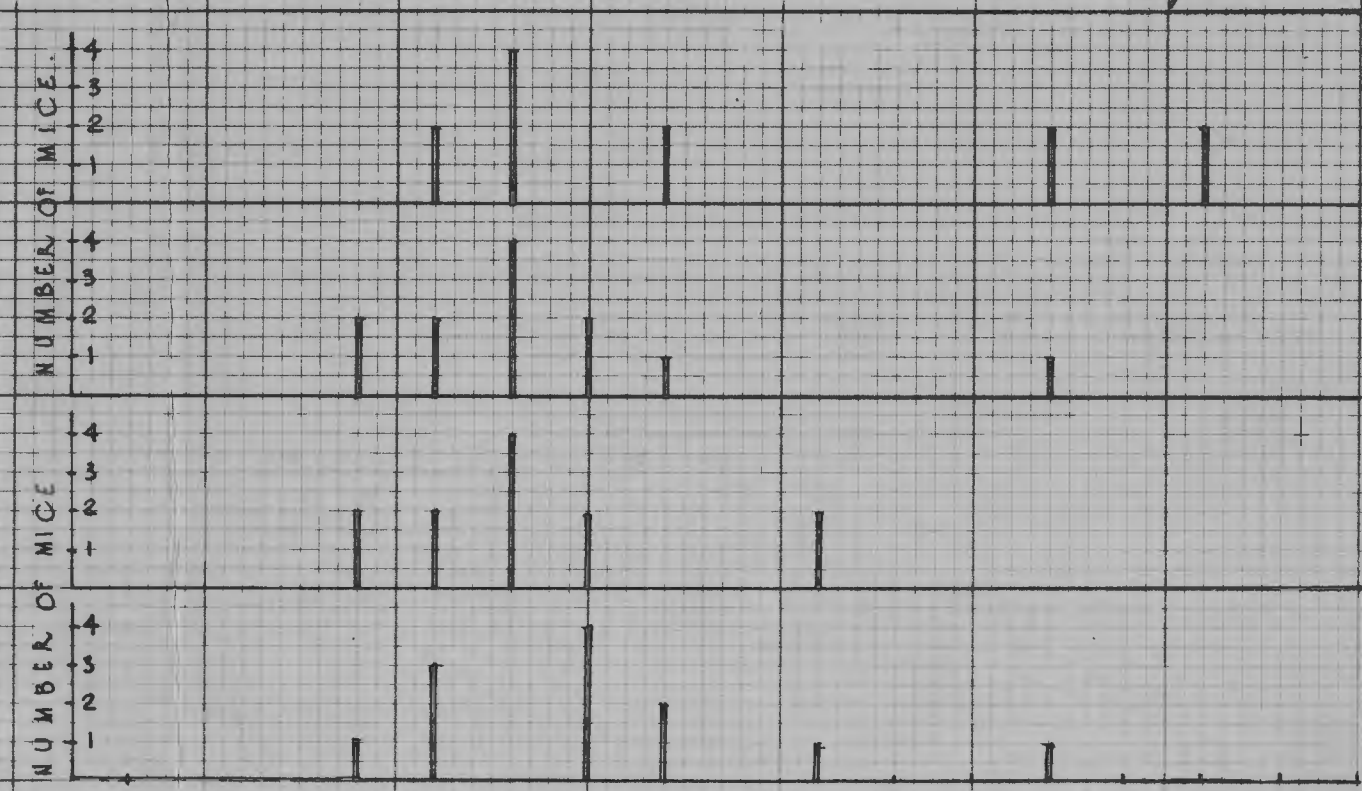


Fig.52 Injection of tetanus toxin 20.0 µg./kg. which resulted in a LD_{100} for the 15th day

such cases no mouse survived the 15th day.

In the first experiment injections of the toxin were given intramuscularly into the hind-leg and 48 mice, separated into 4 groups of 12, were used to establish the LD_{100} for the 5th day. In the second experiment another 48 mice, grouped as in the first experiment, were injected with the toxin LD_{100} for the 5th day diluted with equal parts of 0.9% NaCl. The results of these two experiments are given in Figs. 51 and 52. As indicated in Fig. 52, all the mice died within 15 days.

In the third experiment 36 mice, separated into 3 groups of 12, received the same doses of tetanus toxin as given in the second experiment, but this time together with OES (DP 920). In order to obtain this mixture OES was dissolved in the tetanus/NaCl solution to 4% and it was hoped that the toxin would link on to the OES molecule and thus be very slowly released into the blood stream. Fig. 53 shows that 70% of the mice survived.

In the fourth experiment the same number of mice were used and the same dosage injected as in the third experiment but the tetanus toxin and the OES (DP 920) were injected separately (Fig. 54). 42% of the mice survived.

In the fifth experiment methylcellulose with a DP of 500 was dissolved in tetanus toxin to form a 2%

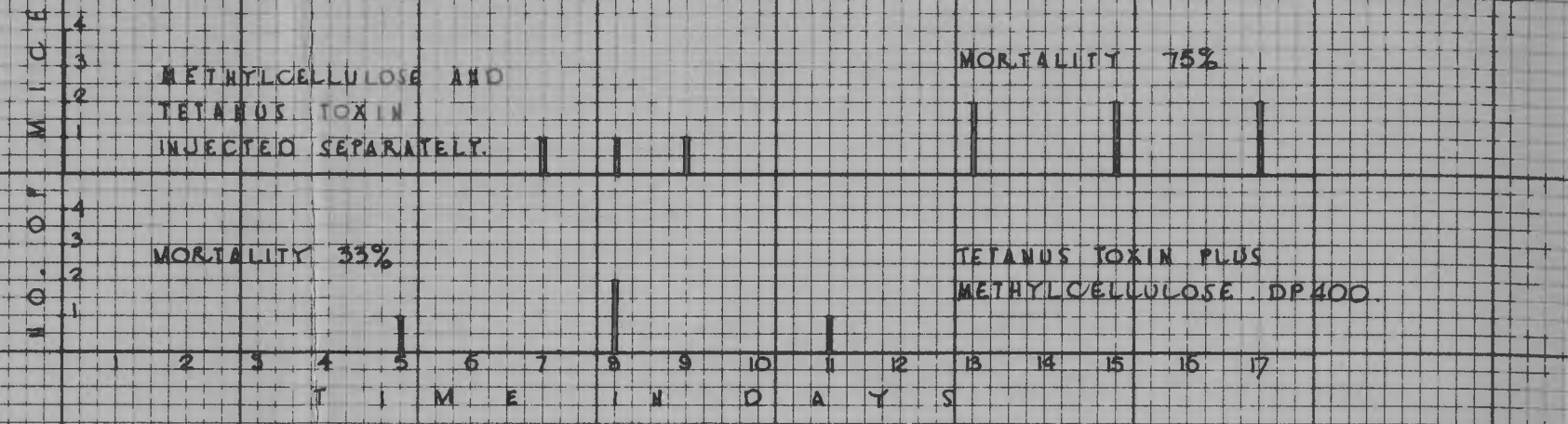


Fig.55 Effect of tetanus toxin and of methylcellulose injected separately and together

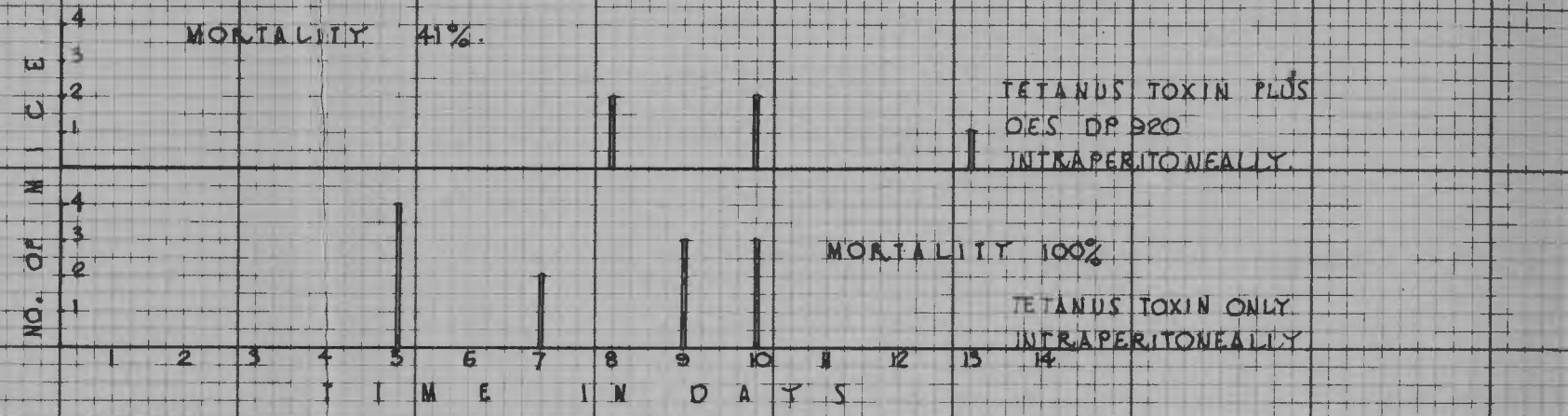


Fig.56 Effect of intraperitoneal injection of tetanus toxin combined with OES and of tetanus toxin only

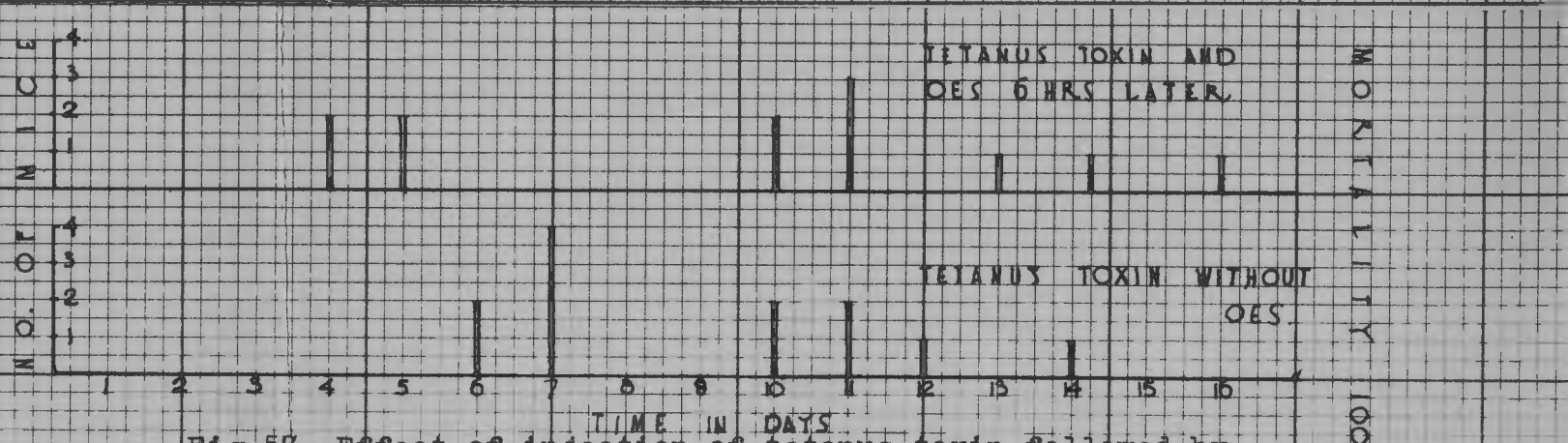


Fig.57 Effect of injection of tetanus toxin followed by an injection of OES and of tetanus toxin injected separately

solution and injected separately into 12 mice. Another 12 mice were injected with the same dosage of tetanus toxin together with methylcellulose. The results in Fig. 55 show that for the separate injections the survival rate was 25% and for the combined injections, 67%. The results were similar to those obtained with separate and combined injections of OES (DP 920) as shown in Figs. 53 and 54.

In the sixth experiment injections were given intraperitoneally into 24 mice. 12 mice received tetanus toxin plus OES (DP 920) and 12 mice received tetanus toxin only. Fig. 56 shows that the tetanus toxin plus OES resulted in a mortality rate of 41%, as against a mortality rate of 100% for the tetanus toxin only.

The seventh experiment shows the effect of OES given 6 hours after an injection of tetanus toxin into the hindleg of each of 12 mice. It had no influence upon the onset and final development of tetanus (Fig. 57) but it was observed that the course of the intoxication was milder and the stiffening of the injected legs less severe. The doses and concentrations of tetanus toxin and of OES (DP 920) were the same as for all these experiments.

DISCUSSION

The many different aspects of the biological action of so varied a group of polysaccharide derivatives makes it difficult to come to a general conclusion.

High polymer substances exerted no marked toxic effect on the tissues. No clear pathological changes could be observed in sections of lung, liver, spleen, and kidney. Neither the blood pressure nor the respiration were affected. The speed of absorption of high polymers, injected subcutaneously, was found to be inversely proportional to the degree of polymerisation of the substances. Strongly heteropolar high polymer substances seemed to be an exception and were found to be very toxic. It remains to be seen whether this toxicity was due to changes in the ionic equilibrium of the blood or to its action on circulatory or respiratory centres. The P A S staining technique indicated that there was a deposition of OES in lung, kidney, spleen, and liver. The fact that OES-^{14}C, injected into rabbits, gave no positive results when β -ray emission of lung tissue was counted, indicated that the β -rays were not recorded by the Geiger-Müller end-window method and that the self-absorption of ^{14}C of the specific activity used was too high or the concentration per

gram tissue too low.

Renal function, tested by measuring the creatinine clearance, decreased after the infusion of OES but recovered later. The fact that the depression of the renal clearance of creatinine was greater when OES of a lower degree of polymerisation (DP) was injected may have been an indication that OES of a higher DP was first trapped in the lung capillaries and then gradually released into the systemic circulation.

Tissue cultures of monkey kidney cells seemed to metabolize OES given in lower concentrations. This is of interest as it opens up new fields in the testing of various high polymers, known as "indigestible", i.e. those which are not broken down by saliva or by pancreatic juice. The pH of the medium which covers the tissue cells decreases when glucose is added: the tissue cells metabolize the glucose, thus lowering the pH. Tissue cells to which OES was given lowered the pH of the medium as well and only the higher concentrations (i.e. the more viscous solutions) killed the tissue cells. No metabolizing activity of the latter was observed. This may have been due to a change of the osmotic pressure within these tissue cells when in contact with the OES solution.

The effect of OES and other high polymer substances used in cell respiration experiments, indicated that

their viscosity as well as their polarity affected the rate of oxygen uptake in liver cells. It is possible that, when put in the Warburg apparatus, the gaseous exchange in the tissues was inhibited by the viscous layer of OES. The heteropolar xylan-sulphuric acid ester and the cellulose-glycolic acid ether also decreased the oxygen uptake although their viscosity is low compared to that of OES. Further experiments in this direction may show whether the viscosity, the shape, or the polarity of the molecule is primarily responsible for the decrease in cell respiration.

The variability of the blood cell counts in rabbits demands a critical approach to all such results. The rapid changes in the number of white cells caused by excitement or pain made it essential to handle the animals with care and a standard technique in taking and counting blood was followed.

The small diameter of the lung capillaries (4-6μ) makes it very probable that macromolecules are blocked when passing through them. Thus, the bigger white cells (granulocytes) cannot pass and consequently their number decreases in the systemic circulation. Lesser degrees of leucopenia were obtained by decreasing the size of linear molecules injected. Leucopenia after injection of OES was also observed when using heart-lung preparations.

This again suggested a direct blocking of OES molecules in the lung capillaries because the leucopenia varied according to the size of the molecule injected. It is possible that the granulocytes, being the larger blood cells, undergo gradual sequestration when trapped in the small lung capillaries. The relative lymphocytosis after injection of linear, high viscous substances was therefore not surprising. The A C T H mechanism appeared to play no part in these experiments as it was unlikely that a few large molecules would stimulate this mechanism more effectively than smaller molecules of the same kind. The results of the heart-lung preparation experiments indicated also that the A C T H mechanism did not alter the white blood cell count.

As it is known that injections of granulocytes increase the body temperature, the increase associated with an injection of linear molecules was probably due to the sequestration of granulocytes in the lung capillaries. In all these experiments it was essential to use pyrogen-free water to dissolve and dilute the substances injected. Contrary to the results of previous experiments by the author, it was shown that branched molecules affected the body temperature of rabbits when injected in a concentration of 10mg./kg. intravenously.

It has been observed that the excretion of OES-^{14}C was faster than expected. The fact that the first fraction of OES-^{14}C, although prepared from a sample of hydrolysed starch with a high DP, degraded so rapidly indicated that all handling of starch in sodium hydroxide solution has to be done in the complete absence of oxygen. Only then can a high polymer fraction be obtained and this remains longer in the blood stream. Both OES fractions used (DP 480 and 900) were of too low a degree of polymerisation to show any difference in the rate of disappearance from the plasma and it seems fair to conclude that OES with a DP of 1,500 or 2,000 would not disappear so rapidly. The disappearance of OES-^{14}C from the blood stream in nephrectomised dogs indicated that there may be many parts of the body in which OES is stored. If it were stored in one or two organs only even the insensitive method of end-window counting would have given an indication of this.

The low β-ray emission of dried or digested organs of the experimental animal did not allow any final conclusion as far as deposition of OES was concerned. Far greater doses or a higher specific activity of ^{14}C should be administered in order to get clear results when using the end-window method. The results of autoradiographic studies which, it was hoped, would give an

indication of the duration and site of OES deposition are still negative. As the exposure-time is long and the β -rays very soft (self-absorption), the first batch developed gave no results. The last batch of still undeveloped autoradiographs which has to be stored in the light-proof container for another 4 months, may give some results. If this longer exposure time does not produce results it may be necessary to use ^{14}C sources with a higher specific activity or to label the starch molecules using ethylene oxide-^{14}C which is by far the best way to produce OES-^{14}C. This method may possibly give more definite results than those obtained in the present investigation. Unfortunately the high cost of ethylene oxide-^{14}C did not allow its use in these experiments.

The toxin-binding properties of OES in methylcellulose are similar to those of polyvinylpyrollidone. These colloidal substances are unlikely to be used therapeutically as the toxin is usually linked to protein-substrates long before the appearance of clinical signs of toxicity. It is, however, doubtful whether the infusion of macromolecular substances will break loose any protein-bound toxin and link it onto the macromolecule. In principle such changes have been observed: Evan's blue, when injected intravenously into dogs, is deposited in the

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body tissues after some of it has been excreted in the urine. An infusion of polyvinylpyrrolidone into dogs previously injected with Evan's blue caused a transference of the dye from the tissues back into the blood stream and from there into the glomerular filtrates and urine. There is little doubt that the effect of the tetanus toxin is lessened once the molecule is linked onto the high polymer. It is also possible that the toxin is linked with its toxophoric group onto the high polymer and in this case the toxic effect observed is due to free tetanus toxin which has not been linked onto the macromolecule.

The practical application of this anti-tetanus therapy has so far yielded no encouraging results. On the other hand, the relief of spasm and suffering reported justifies its continued use.

SUMMARY

1. The concept of macromolecules is discussed and their use in medicine described.

2. The relation between biological actions of some high polymer carbohydrate derivatives and their chemical structures are discussed.

3. The production and labelling of oxyethylstarch and oxyethylstarch-^{14}C are described.

4. Investigations on the toxicity of some carbohydrate derivatives, especially oxyethylstarch, were carried out. It was shown that none of these substances except xylan and cellulose-sulphuric acid esters of a high molecular weight were of acute toxicity. Respiration and blood pressure were not affected by polysaccharide derivatives, whether linear or branched, charged or uncharged; an exception to this was the cellulose-sulphuric acid ester of high molecular weight.

5. Experiments to show the effect of oxyethylstarch on monkey tissue cells indicated that the high fraction (DP 2,000) affected the normal growth of cells when the

solution added to the medium was given in 1% or higher concentrations. A fraction with a low degree of polymerisation (170) also influenced the growth of tissue cells but to a lesser degree. Dextran given in the same concentrations had no inhibiting effect on tissue cells.

6. Tissue respiration experiments showed that the low fraction of oxyethylstarch (DP 170) did not inhibit the oxygen uptake of liver cells whereas methylcellulose of medium and oxyethylstarch of a high degree of polymerisation (DP 500 and 2,000 respectively) inhibited the oxygen uptake. Xylan-sulphuric acid ester and cellulose-glycolic acid ether also inhibited the oxygen uptake of liver homogenates.

7. Renal clearance tests indicated that infusion of oxyethylstarch of a low degree of polymerisation (DP 300) decreased the renal clearance for creatinine during the time of infusion and for a short while thereafter.

8. The histological findings after prolonged administration of oxyethylstarch showed little or no change in the organs examined but certain non-specific

reactions such as swelling and congestion could be seen. This has been reported in similar experiments with dextran and polyvinylpyrrolidone.

9. The effects of high-polymer substances on the erythrocyte sedimentation rate, body temperature, and white cell count is described. The erythrocyte sedimentation rate was influenced by the polarity and length of the molecules investigated. The body temperature was increased by linear and, to a lesser extent, by branched molecules of homopolar character. Homopolar polysaccharide derivatives of linear or branched structure evoked a leucopenia with specific granulocytopenia causing a relative lymphocytosis.

10. The use of oxyethylstarch as a blood volume expander was demonstrated. There is little difference between the results of experiments carried out with dextran and those using oxyethylstarch. However, the comparatively low cost of producing standardized oxyethylstarch is an advantage. Another advantage is that, in the production of oxyethylstarch, the degradation of the raw material can be controlled as it is not dependent on bacterial activity.

11. The distribution and excretion of oxyethylstarch showed that the substance is readily excreted in the urine. It was not stored in one or two organs only but was apparently widely distributed; it is possible that selfabsorption was too high to show clear results when the β -ray emission of these organs was measured with an end-window device which is not highly sensitive.

12. The toxin-binding properties of oxyethylstarch were investigated. When tetanus toxin with an $L.D._{100}$ for the 15th day was combined with oxyethylstarch and injected together with it the toxicity of the tetanus toxin decreased and only 30% of the mice died within 15 days as compared with 100% in the control experiments using pure tetanus toxin.

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SUMMARY

1. The concept of macromolecules is discussed and their use in medicine described.

2. The relation between biological actions of some high polymer carbohydrate derivatives and their chemical structures are discussed.

3. The production and labelling of oxyethylstarch and oxyethylstarch-^{14}C are described.

4. Investigations on the toxicity of some carbohydrate derivatives, especially oxyethylstarch, were carried out. It was shown that none of these substances except xylan and cellulose-sulphuric acid esters of a high molecular weight were of acute toxicity. Respiration and blood pressure were not affected by polysaccharide derivatives, whether linear or branched, charged or uncharged; an exception to this was the cellulose-sulphuric acid ester of high molecular weight.

5. Experiments to show the effect of oxyethylstarch on monkey tissue cells indicated that the high fraction (DP 2,000) affected the normal growth of cells when the solution added to the medium was given in 1% or higher concentrations. A fraction with a low degree of polymerisation (170) also influenced the growth of tissue cells had no inhibiting effect on tissue

6. Tissue respiration experiments showed that the low fraction of oxyethylstarch (DP 170) did not inhibit the oxygen uptake of liver cells whereas methylcellulose of medium and oxyethylstarch of a high degree of polymerisation (DP 600 and 2,000 respectively) inhibited the oxygen uptake. Xylan-sulphuric acid ester and cellulose-glycolic acid ether also inhibited the oxygen uptake of liver homogenates.

7. Renal clearance tests indicated that infusion of oxyethylstarch of a low degree of polymerisation (DP 300) decreased the renal clearence for creatinine during the time of infusion and for a short while thereafter.

8. The histological findings after prolonged administration of oxyethylstarch showed little or no change in the organs examined but certain non-specific reactions such as swelling and congestion could be seen. This has been reported in similar experiments with dextran and polyvinylpyrrolidone.

9. The effects of high-polymer substances on the erythrocyte sedimentation rate, body temperature, and white cell count is described. The erythrocyte sedimentation rate was influenced by the polarity and length of the molecules investigated. The body

temperature was increased by linear and, to a lesser extent, by branched molecules of homopolar character. Homopolar polysaccharide derivatives of linear or branched structure evoked a leucopenia with specific granulocytopenia causing a relative lymphocytosis.

10. The use of oxyethylstarch as a blood volume expander was demonstrated. There is little difference between the results of experiments carried out with dextran and those using oxyethylstarch. However, the comparatively low cost of producing standardized oxyethylstarch is an advantage. Another advantage is that, in the production of oxyethylstarch, the degradation of the raw material can be controlled as it is not dependent on bacterial activity.

11. The distribution and excretion of oxyethylstarch showed that the substance is readily excreted in the urine. It was not stored in one or two organs only but was apparently widely distributed; it is possible that selfabsorption was too high to show clear results when the β -ray emission of these organs was measured with an end-window device which is not highly sensitive.

12. The toxin-binding properties of oxyethylstarch were investigated. When tetanus toxin with an $L.D._{100}$ for the 15th day was combined [illegible] days as compared with 100% in the control experiments using pure tetanus toxin.