CXCIII. THE INFLUENCE OF COD-LIVER OIL ON THE HAEMOLYTIC COMPLEMENT OF HUMAN BEINGS.

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IN a previous communication [Osborn, 1931], it was shown that the complement of rats fed on a diet deficient in vitamin A tended to be lower than that of control animals receiving cod-liver oil. The aim of the present work was to discover whether a dose of cod-liver oil produced a change in complement concentration in human beings. Fifty cases were experimented on, and they were compared with fifty cases receiving olive oil. A large dose of 50 cc. was given in each case in order to obtain the maximum possible effect. This was taken without difficulty by all the subjects except one. A Zulu who had a superstitious objection to fish of any sort and who detected the fishy flavour of the cod-liver oil vomited a few cc. of his dose.

In all 100 experiments were made:

- 14 on Oxford students and research workers,
- 24 on South African students,
- 42 on natives in the Johannesburg Hospital,
- 20 on natives in the City Deep Mine Hospital.

Fourteen individuals submitted to both the cod-liver oil and the olive oil, with an interval of a day or more between the experiments. In the other experiments alternate individuals were given cod-liver oil and olive oil respectively. The students were all normal healthy individuals. The hospital cases were convalescents, usually a day or two before their discharge, fractures and other simple surgical cases uncontaminated by sepsis, and others similarly free from any acute infection.

In each experiment about 0.3 cc. of blood was withdrawn from the finger tip into a small agglutination-tube between 8 and 10 a.m. The dose of oil was then given and one or more further samples of blood were taken from one to five hours after the dose.

The tubes were stoppered and allowed to stand at room temperature for 24 hours. The complement activity of the resulting serum was then estimated by measuring the time taken for complete haemolysis of a fixed volume of sensitised sheep's corpuscles by a measured volume of the serum. Two observations were made on each sample of blood. The S.A. Institute for Medical Research kindly arranged for a regular supply of the corpuscles. They were sensitised with five units of anti-sheep corpuscle serum and were suspended in normal saline to give a 3 % suspension. The details of their preparation are as follows.

2 parts of sheep's blood are drawn into 1 part of a 2 % solution of sodium citrate. The corpuscles are deposited by centrifuging and are washed three times

with 3 volumes of 0.85 % NaCl, being centrifuged for 10 minutes at 3000 r.p.m. between each washing. A volume of corpuscles, measured by means of a pipette, is then suspended in sufficient 0.85 % saline to give a 3 % suspension. To this are added 5 units of anti-sheep corpuscle serum (*i.e.* five times the minimum volume needed for complete haemolysis in the presence of complement). The mixture is then allowed to stand for at least an hour.

A capillary pipette described in the Appendix was used for the measurements. The following volumes were drawn up in the order stated with a bubble of air between each:

1.0 vol.	normal saline
1.0 „	normal saline
x „	serum diluted three or four times with normal saline
$1-x$ „	normal saline
0.5 „	normal saline
0.5 „	3 % suspension sensitised sheep's corpuscles
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Total	4.0 vol.

In the pipette used 1 volume was equal to about 50 mm.³ Since all the measurements were made with the same pipette, the absolute volume is of no importance. The measured volumes were expelled in the opposite order into an agglutination-tube. This procedure ensures that the serum is washed out of the pipette by two volumes of saline, and that it is separated from the sensitised corpuscles by two small volumes of saline. The solutions were mixed thoroughly; and the tube was warmed in a glass-sided water-bath at 37°. A white glazed tile, ruled with black lines, was placed behind the tubes in the bath, and they were illuminated with a strong beam of light. In this way it was possible to estimate, to within a few seconds, the point of complete haemolysis. Since a reaction taking longer than about 4 minutes has a less definite end-point, the dilution of the serum and the volume of the dilute serum used (x in the list of reagents), were adjusted to give a haemolysis time of 2-3 minutes.

The activity of the complement after the dose was expressed as a percentage of the strength before the dose.

$$\begin{aligned}
 & \frac{\text{Activity of complement after dose}}{\text{Activity of complement before dose}} \times \frac{100}{1} \\
 = & \frac{\text{Speed of haemolysis after dose}}{\text{Speed of haemolysis before dose}} \times \frac{100}{1} \\
 = & \frac{\text{Time for complete haemolysis before dose}}{\text{Time for complete haemolysis after dose}} \times \frac{100}{1}.
 \end{aligned}$$

For these times to be comparable it was obviously necessary for the same volume of serum to be used in the estimation of the activity before and after the dose, *i.e.* in any one comparison the dilution of the serum and the volume of the diluted serum (x) were the same.

In a few of the cases samples of blood were taken hourly from 1 to 5 hours after the dose, in others a single sample was taken at from 2½ to 4 hours. In all 174 estimations were made following cod-liver oil and 170 following olive oil. The results are expressed in the form of a histogram in the accompanying diagram. 100 %, marked by the heavy black line, is the strength of the complement before the dose.

Protocol of one pair of observations:

Subject: J. H. G.

Date: 18th Jan. 1933.

Blood sample taken at 9.30 a.m., dose of 50 cc. of cod-liver oil given immediately after, second sample taken at 1 p.m., food and violent exercise being avoided in the interval. Blood stood at room temperature overnight to allow separation of the serum. Serum diluted with 3 volumes of 0.9 % saline.

	9.30 a.m. blood	1 p.m. blood
Volume diluted serum	0.6	0.6
Volume sensitised corps.	0.5	0.5
Volume 0.9 % saline	2.9	2.9
Haemolysis time	168 secs.	145 secs.
Percentage of original speed	—	$\frac{168 \times 100}{145} = 116 \%$

Fig. 1. Histogram showing complement activity of serum of individuals after taking cod-liver oil and olive oil. Expressed as percentage of activity of serum taken before dose.

The statistical analysis of the figures gives the following results:

Mean of cod-liver oil	= 113.4
Mean of olive oil	= 102.7
Difference	= 10.7

Standard deviation of cod-liver oil ($\sigma_{C.L.O.}$) = 19.0

Standard deviation of olive oil ($\sigma_{O.O.}$) = 10.8

Standard error of difference ϵ_{12}

$$= \sqrt{\frac{\sigma_{C.L.O.}^2}{N_{C.L.O.}} + \frac{\sigma_{O.O.}^2}{N_{O.O.}}} = 1.66.$$

Since the deviation of the means is approximately 6.5 times the standard error of the difference, it seems to be significant.

DISCUSSION.

It is an accepted fact that haemolytic complement is not a single substance. The work of Buchner [1893], Ferrata [1907], Sachs and Teruuchi [1907], Brand [1907], and others showed that it contained two thermolabile parts, the "Midstuck" and "Endstuck." The work of v. Dungern [1900], Ehrlich and Sachs [1902], Coca [1914], Hyde [1925] and others led to the recognition of a third part, while Gordon, Whitehead and Wormald [1926] produced good evidence of a fourth part. In these circumstances it would not be expected that an increase in the concentration of one of the parts would invariably lead to an increase in the total complement activity. This would only occur if the part increased were the limiting factor in the blood in question. This supposition may explain the unevenness of the results obtained. While the administration of cod-liver oil caused an increase as compared with the olive oil when measured by the average effect, and while this increase was well marked in a percentage of cases, it did not occur invariably. This is what might be expected by our previous reasoning. If some factor, present in cod-liver oil and absent from olive oil, caused an increase in one of the parts of the complement complex, an ingestion of complement would cause an increase in the total complement activity in some cases but not in others.

These results are interesting when taken in conjunction with the previous results [Osborn, 1931], which showed that rats fed on a diet deficient in vitamin A had, on the average, a lower complement than control rats receiving cod-liver oil.

SUMMARY.

The ingestion of a dose of cod-liver oil by 50 experimental subjects produced, on the average, a rise in haemolytic complement which was greater than that produced by olive oil. The significance of the fact that this rise took place in some cases but not in others is discussed.

The author's thanks are due to Prof. J. P. Dalton, who kindly undertook the statistical analysis of the results, to Dr G. Buchanan of the S.A. Institute for Medical Research for the supply of blood corpuscles, and to the subjects who allowed a rather unpleasant experiment to be performed on them.

APPENDIX.

The instrument described below has been found to be suitable for the repeated measurement of volumes of liquid of the order of 5 mm.³

A piece of tubing is drawn out into the shape shown in Fig. 2. A small drop of mercury, equal in volume to the smallest graduation required, is introduced at the broad end *D* and allowed to run round into the capillary *A*. A rubber teat is filled with mercury and pushed over the broad end *D*. Pressure on the teat is regulated by means of a screw-clip and in this way the column of mercury in the capillary *A* may be moved down the tube and the required number of graduations marked on the tube in Indian ink or glass paint. The instrument is then clamped in a vertical position. A second clamp holds the rubber teat. Liquids are drawn into the pipette by relaxing the pressure on the teat and expelled from it by increasing the pressure.

For accurate measurement the elasticity of the air within the instrument is a disadvantage and therefore the volume of this air is reduced as much as possible. It is for this reason that *C* is drawn out into a capillary and that the teat is filled with mercury.

In making mixtures of different volumes a method is used which is described by Wright. The measured volumes are separated by columns of air in the capillary. If, for instance, it is required

to mix 1.7 volumes of *A* with 0.6 volumes of *B* by means of a pipette holding 1 volume and graduated into 10 divisions, the following are drawn up into the pipette:

1.0 volumes *A*,
a small column of air,
0.7 volumes *A*,
a small column of air,
0.6 volumes of *B*.

These are then all expelled together. They may be mixed by being repeatedly drawn in and out of the pipette. Another convenient method is to blow a small bubble of air into the mixture and rotate it about.

The small bulb *B* prevents liquids being drawn over into the mercury in *D*.

Fig. 2. Diagram of pipette.

Wright [1921] advocates the coating of the inside of small pipettes with wax to obviate the error produced by wetting the glass. The author has not, up to the present, found a wax which will hold to the walls for any length of time. If such a substance could be found, it would be a great advantage in all volumetric micro-chemical work. An alternative suggestion of Wright's has, however, been found to be helpful. If the external surface of the tip of the pipette has a coating of wax the removal of the last drop of liquid is facilitated.

In most cases one pipette would be used for all measurements, therefore the absolute volume of the graduated part of the pipette need seldom have any definite value. Provided it is divided into a number of equal parts, relative volumes can be measured accurately.

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