A PRELIMINARY STUDY OF THE BIO-CHEMICAL RELATIONS OF VARIOUS LIPOID SUBSTANCES IN THE LIVER

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The discovery of Landsteiner, Müller and Pötzl that an alcoholic extract of human or animal liver could be used as an antigen, shed quite a new light on the Wassermann reaction. The idea that this reaction was of the same nature as the typical Bordet-Gengou phenomenon had to be abandoned. An enormous amount of work on the subject at once followed, and it was shown that the test could be performed with extracts of other organs, as well as with substances such as lecithin, cholesterol, bile salts and phosphatides from different organs.

Further, it was found that the antigenic properties of these substances varied considerably, and their suitability for use was still further modified by other factors, such as their haemolytic properties. While most workers with the Wassermann reaction have been content to use alcoholic extracts of liver or heart of varying composition, which were rejected if they possessed undesirable properties, others have made efforts to apply substances of more or less definite nature and free from the most objectionable features such as the presence of protein or haemolytic bodies. These have chiefly been lecithides obtained from various organs. Browning used a lecithin obtained by digesting a heart with ethyl acetate at 60° C., precipitating with acetone, and further purifying by repeatedly taking it up with ether and precipitating with acetone. He found that the antigenic powers of such a lecithin are increased by the addition of cholesterol.

Noguchi has advocated the use of a lecithin obtained by treating a liver or heart with warm alcohol, evaporating the extract to dryness by means of a current of air at 37° C., and treating this repeatedly with ether to separate the ether-soluble substances. The ether-soluble portion is evaporated, then dissolved with a little ether and precipitated with water-free acetone. It is this acetone-insoluble fraction which Noguchi has recommended for use as antigen.

Recently, Noguchi and Bronfenbrenner have published the results of an investigation into the antigenic, anti-complementary, and haemolytic properties of the different ether and acetone soluble and insoluble fractions obtained in the above process. The livers were chiefly derived from human beings who had suffered from various disorders, but, in addition, the livers of animals were also examined. The iodine values of the acetone-insoluble fractions were determined, and an attempt made to associate these with the antigenic properties and the clinical conditions. These investigators found that the most satisfactory antigenic properties were shown by the acetone-insoluble fractions and that, speaking generally, a high antigenic value in these fractions was associated with a high iodine value. This group consists chiefly of phosphatides.

Some recent workers on lipoids, such as Erlandsen and Maclean, have thought that under certain conditions part of these bodies might be in a free state in the tissues, and the rest combined with other tissue elements from which they might be separated by suitable treatment. The present investigation was undertaken with the object of ascertaining the haemolytic and anti-complementary properties of extracts obtained by the method used by these workers.

The tissue was first extracted with ether, then with cold alcohol, and finally with hot alcohol. From these three main extracts further fractions were separated by evaporation and precipitation with acetone. If the amount of material in each fraction sufficed, the saponification and iodine values were worked out.

METHOD OF PREPARATION

A pig's liver, obtained immediately after the slaughter of the animal, was passed through a mincing machine, and then spread out on glass plates and dried under a fan at 30° C. The dried mass was passed through a coffee mill and the resulting fine powder thoroughly dried *in vacuo* over H_2SO_4 .

The tissue was then extracted with ether in a shaking machine for three or four hours and rapidly filtered. This was repeated four times till the addition of acetone caused only a very faint cloudiness. This extract was carefully preserved in a stoppered bottle in the ice-chest.

The residue of the tissue was then repeatedly treated with cold absolute alcohol in a similar manner, and the extract preserved. Finally,

the tissue was extracted with hot absolute alcohol until practically no more acetone-insoluble material could be obtained.

Three different extracts were thus obtained, which were treated in the following manner:—

Ether-soluble Extract.—The ether was distilled off at 40° C. until the extract had a syrupy consistency. Excess of acetone was now added, when a brownish-yellow mass separated out. The residual fluid was filtered and a clear yellow fluid obtained which, on evaporation under the fan, gave a greasy-looking yellow substance. These two fractions were preserved over H_2SO_4 *in vacuo*.

Cold Alcohol Extract.—Evaporation under the fan yielded a grayish greasy-looking substance. This was thoroughly extracted with ether and two fractions thus obtained—one ether-soluble and the other ether-insoluble. The former was further separated into an acetone-soluble portion of white colour, and a brownish acetone-insoluble one. Only very small amounts of these two fractions were obtained, as most of the cold alcohol extract seemed to be insoluble in ether, and was obtained as a light brown tenacious substance.

Hot Alcohol Extract.—On leaving this in the ice-chest over night, a small quantity of a brownish-yellow substance was deposited. This was insoluble in hot alcohol, but partly soluble in ether, from which a white precipitate was thrown down by acetone. This acetone precipitate was found to contain a fair amount of phosphorus. The rest of the hot-alcohol extract was evaporated until a dark brown sticky mass was left. This was treated in a similar manner to the cold alcohol extract. The ether-soluble fraction thus obtained was very small in amount and was practically all soluble in acetone. The various fractions thus obtained were preserved over H_2SO_4 in exhausted amber-coloured desiccators, and their haemolytic and anti-complementary properties tested as soon as possible

Preparation of Emulsions.—In each case the emulsion was prepared by dissolving 0.025 gram of the substance in a little warm absolute alcohol, and slowly adding 5 c.c. of a 0.9 per cent. solution of sodium chloride.

Determination of the Haemolytic Property.—Increasing dilutions of the emulsions were made with 0.9 per cent. saline solution. Four parts of each of these dilutions were mixed in a series of test-tubes with one part of a 20 per cent. suspension of washed human red blood corpuscles. The tubes were placed in a water bath at 37° C., and the

result noted after incubation for two hours. The figures in the accompanying table denote the weakest emulsion which produced haemolysis.

Determination of the Anti-complementary Property.—The technique used for this purpose was that advocated by D'Este Emery. This worker has pointed out in his Hunterian Lecture that all sera will give some absorption of complement if the extract be strong enough, and that the difference in the powers of absorption between normal and syphilitic sera is only one of degree. He claimed that for standardizing an extract it was better to use normal sera, as these are very much more constant than syphilitic ones, which vary over a wide range. This plan has been adopted in the present investigation, and the absorptive powers of the extracts tested against normal sera.

Progressing dilutions of each emulsion were placed in a series of small test-tubes and mixed with fresh normal human serum in the proportion of four parts of the former to one of the latter. The tubes were then placed in a water-bath at 37° C. for half an hour. To each tube was now added one part of a 20 per cent. suspension of sensitized human red blood corpuscles, which had previously been saturated with an excess of an appropriate dilution of anti-human rabbit serum. By using this technique of D'Este Emery a very minute amount of unabsorbed complement may be detected. Moreover, the time taken up is very much lessened, as haemolysis occurs very rapidly when there is any free complement, and when absorption has been complete the agglutination of the corpuscles enables the result to be read off in a very short time. The figures in the chart denote the weakest dilution which gave complete absorption.

Fraction				Saponification value	Iodine value	Haemolytic property	Anti- complementary value
Ether-soluble—							
1.	Acetone-soluble	...	...	190.5	106.5	2	2
2.	Acetone-insoluble	...	...	252	92.4	4	72
Cold alcohol—							
1.	Entire	...	...	246.4	28.4	9	22
2.	Ether-insoluble	...	...	285.5	16.9	8	5
3.	Ether-soluble—						
	(a)	Acetone-soluble	...	—	47.5	5	38
	(b)	Acetone-insoluble	...		Amount insufficient.		
Hot alcohol—							
1.	Entire	...	...	266	54	8	6
2.	Ether-insoluble	...	...	257	9.1	7	31
3.	Ether-soluble—						
	(a)	Acetone-soluble	...	—	32.2	5	20
	(b)	Acetone-insoluble	...		Amount insufficient.		

CONSIDERATION OF RESULTS

Saponification Values.—In the cases where sufficient material was obtained to estimate them, there was not much variation in the saponification values, except in the acetone-soluble fraction of the ether extract which was somewhat lower than the others. This may have been due to its containing fats of a higher molecular weight or to the presence of cholesterin.

Iodine Values.—The ether extract showed the greatest degree of unsaturation; the acetone-soluble fraction, as might be expected, giving the highest iodine value. The other extracts gave considerably lower values; that of the cold alcohol being about half the hot alcohol figure. No relation between the iodine value and the haemolytic property could be established. The fact that the acetone-soluble fraction of the ether extract, with its high iodine value, was practically non-haemolytic may have been due to the presence of cholesterin.

Haemolytic Property.—The fractions derived from the ether extract had a much weaker haemolytic action than those of the other extracts, which did not differ much from each other.

Anti-complementary Property.—Great differences were manifested by the various fractions in this respect. The acetone-soluble portion of the ether extract had practically no power of absorbing complement. On the other hand, the acetone-insoluble fraction possessed this property to a very high degree, in fact, was two and a half times stronger than the next highest fraction. A curious difference was shown by the cold and hot alcohol extracts, and the fractions obtained from them. While the entire cold alcohol extract gave a value of 22, and the hot alcohol a value of 6, the ether-insoluble fraction of the latter yielded a value of 31 against a value of only 5 by the ether-insoluble fraction of the former. The acetone-soluble fractions of the ether-soluble portions of both cold and hot alcohol extracts possessed very fair powers of absorbing complement, but, as mentioned above, were much inferior to the acetone-insoluble portion of the ether extract.

It was unfortunate that the amount of liver originally used was insufficient to yield enough acetone-insoluble portions of the cold and hot alcohol extracts to perform the tests. The greatest amount of material was obtained from the ether extract, which also contained a large quantity

of acetone-insoluble substances. Very small amounts of the cold and hot alcohol extracts were soluble in ether, and the greater part of what was soluble was also soluble in acetone.

CONCLUSIONS

1. The acetone-insoluble fraction of the ether extract yields the most suitable antigen.

2. The cold and hot alcohol extracts yield substances differing from each other and from the ether extract in physical and bio-chemical properties.

3. These differences are apparently not dependent on the saponification or iodine values.