CXXXIV. THE FAT, LIPIN AND CHOLESTEROL CONSTITUENTS OF ADRENALS AND GONADS IN CASES OF MENTAL DISEASE.

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THE late Sir Frederick Mott, who suggested and initiated this work, stated that in his opinion, the fat, cholesterol and lipin substances of the nervous system and of the gonads were possibly manufactured in the cortex of the suprarenal gland, from which the material was carried by the blood for the nutrition of the gonad and developing brain. The present work was therefore undertaken upon his suggestion with the object of showing the possible relations between the suprarenal and gonad fatty constituents and their relative proportions in cases in which mental disorder is a marked feature. There are often marked histological changes in the gonads in cases of mental disorder and therefore such balance as possibly exists in the normal is most likely to be disturbed in these cases.

The adrenal cortex is essential to life in the higher animals. The experiments of Biedl [1910] show that the lipin-containing cortex is even of more vital importance than the medulla. Chauffard, Laroche and Grigaut [1920] found that the cholesterol content of the normal gland was as much as 5.5 % of the fresh tissue, *i.e.* twice that of any other organ. The cortex cells of the healthy adrenal are almost filled with fatty constituents of which cholesterol and doubly refracting lipins form the greater proportion. The disappearance of a large amount of these constituents in acute infection, muscular exhaustion and many chronic diseases, suggests that it is the deficiency consequent upon the exhaustion of these vital materials which causes death, and the condition is probably parallel to that found in animals after adrenalectomy.

There is little agreement, however, among various investigators regarding either the function of the adrenal in fat metabolism, or its relationship to the sex glands and brain tissue.

Mott and his collaborators [1917, 1920, 1923], and other workers, have found that morbid conditions of the sex glands were often accompanied by deficiency or atrophy of the adrenal cortex. Developmentally the gonads and adrenals are associated, since both are formed in the embryo from similar and proximate mesoderm cells of the genital ridge. A physiological association probably continues throughout life.

In animals where sexual activity varies with the season of the year, there

is a corresponding increase in the size and lipin content of the adrenal parallel with the sex gland activity. It has been claimed by Lespinasse that precocious sexual development was experimentally produced in a cockerel by transplantation of adrenals. Somewhat analogous changes occur, simultaneously with changes in the ovary, in the cortex cells of the adrenal during the oestrous cycle, and enlargement of the cortex and increase in cholesterol take place during pregnancy (Grigaut).

Glynn suggests that the adrenal controls the development and differentiation of the sex cells. Elliott however asserts that the adrenal is chiefly associated with the muscular system. Chauffard [1918] has shown that the cholesterol content of the foetal adrenal increases greatly after the fifth month.

The adrenal of the human foetus is relatively large, due to a wide "boundary zone" [Elliott and Armour, 1911]. Although this zone is not particularly rich in lipin before birth, later its cells become so filled with doubly refracting fatty constituents that it is with difficulty distinguishable as a separate zone.

In the anencephalic foetus this particular area is absent and the gland resembles that of other foetal mammals, which further emphasises the possibility of a connection between the adrenal cortex and the developing brain.

The suggestion that the adrenal synthesises cholesterol was put forward by Chauffard but the experiments of Bauman and Holly [1923] and more recently of Randles and Knudson do not support this.

Analyses of the cholesterol and lipin content of the adrenal and sex glands have been carried out by a number of workers. The figures for cholesterol in the human adrenal obtained by Chauffard and other French investigators range from 0.2 % to 9.0 % for fresh tissue and illustrate the great variations which may occur in the amounts present; they suggest that this variation is chiefly dependent upon the cause of death, the lowest contents being found after septic conditions such as tuberculosis or septicaemia.

In the present investigation the following constituents were separately determined: lecithin (including kephalin), sphingomyelin, neutral fat, fatty acids, cholesterol ester and free cholesterol. The quantity of lecithin was checked by phosphorus estimation and the neutral fats were found both directly from the fatty acid and by difference from total extract.

METHODS.

Preparation of the tissue. The glands were collected as soon as possible after death, carefully dissected free from fat and fibrous tissue and weighed. Small portions were preserved for histological examination and the remainder was finely minced. The testis was freed from the epididymis and capsule. The cortex and medulla of the adrenal were not separately investigated. The minced glands were preserved and fixed in 30 cc. of freshly prepared 3 % metaphosphoric acid. This reagent coagulated the proteins thus rendering the fatty constituents more accessible to the solvent action of the extracting fluids. After 24 hours the aqueous fluid was removed and discarded, the tissue washed

with water and carefully dried with bibulous paper. 5 cc. per g. of a mixture of alcohol and ether (2 : 1) were then added; the tissues could be preserved indefinitely under these conditions. For estimation the solvent was decanted and evaporated, and the remaining tissue carefully dried by a current of warm air. The residue was ground to a fine powder and dried in a desiccator after which the remaining fatty material was extracted by the special methods given below.

Extraction of the dried tissue. Owing to the difficulty of complete extraction of the tissues, it was necessary to devise a simpler and more rapid method of extracting the fats. This was effected by four successive digestions with alcohol and ether in simply constructed bombs at 150° . A sketch of the bomb employed is given in Fig. 1, the length of the tube being 4.5 inches and the internal diameter 1 inch.

Fig. 1. Bomb for extraction of dry tissue.

The dotted, shaded portions indicate soft solder which forms an effective seal without the use of any washer whatsoever. Each bomb was tested in boiling water using ether.

The solutions obtained by this method of extraction were combined, evaporated and the fat added to that obtained by evaporating the solvent previously decanted from the tissues. The whole was then dissolved in a small volume of chloroform and the slight residue of non-fatty extractive discarded. In order to remove any phosphoric acid which might possibly be retained from the fixing fluid, about 0.1 g. of barium carbonate was added to the chloroform solution which was allowed to stand 2 hours with occasional rotation and then filtered; the residue was thoroughly washed with chloroform, and the washings were added to the extract. (The residue was decomposed with 20% hydrochloric acid to liberate the fatty acid which was estimated separately.) The purified extract was again carefully evaporated, thoroughly dried, weighed, and dissolved in a fixed volume, usually 25 cc., of alcohol containing a small proportion of chloroform. Suitable quantities were then taken for investigation of the various constituents as follows.

ESTIMATION OF THE CONSTITUENTS OF THE EXTRACT.

A. *Lipins containing phosphorus.*

10 cc. of the solution were evaporated and treated with 10 cc. pure acetone. The flask was occasionally rotated for 2 hours. The solution was then decanted for future examination and the operation repeated¹. The acetone-insoluble phosphatides were next dissolved in a small quantity of ether and reprecipitated with acetone. This was decanted and mixed with the above soluble fraction. Three similar precipitations were necessary owing to the solubility of the lecithins in the fatty mixtures. The final residue consisted of mixed lipins containing phosphorus from which the sphingomyelin was separated by first extracting with hot alcohol and then allowing the mixture to cool, the sphingomyelin being precipitated. The precipitate was washed with ether and the phosphorus estimated as below. The remainder of the lipins containing phosphorus, consisting of lecithin and kephalin, was obtained by evaporating the alcohol. It was weighed and then the amount of phosphorus determined as below.

Phosphorus estimation. About 0.1 g. of the lipin was transferred to a small nickel crucible, and for each 0.05 g. of phosphatide 0.5 cc. of a saturated solution of magnesium nitrate and about 0.02 g. of magnesium oxide were added, the whole being allowed to evaporate slowly on a hot plate. The crucible was then heated with a Bunsen burner, at first cautiously, then strongly.

The ash was dissolved in 5 cc. of 50 % nitric acid and 15 cc. of 34 % ammonium nitrate were added, the solution heated to 100° and the phosphate precipitated with 10 cc. of 3 % ammonium molybdate. After standing for 12 hours the supernatant liquid was decanted through a filter, the phosphomolybdate washed with dilute nitric acid and dissolved in 10 cc. of 2 % ammonia. After transferring to a clean vessel, adding 0.5 cc. of molybdate solution and heating to 100°, 5 cc. of 10 % nitric acid were poured in slowly. The ammonium phosphomolybdate was reprecipitated, collected on a Gooch crucible, washed, dried and weighed. The factors by which the weights of the molybdate precipitate were multiplied were: for phosphorus, 0.0163; for lecithin (m.w. 770), 0.405; for sphingomyelin (m.w. 750), 0.396.

Sphingomyelin contains 43 % of lignoceric acid [Levene, 1916]; and the lecithin about 72 % of stearic, palmitic and linolic acids. These figures were used in calculating the lipin and fatty acids which appear as a portion of the total fatty acids estimated below.

¹ The solutions decanted were evaporated, the residue was dissolved in a small quantity of ether, acetone added and allowed to stand several hours or overnight. The precipitate, if any, was added to the phosphorus-containing lipins and the supernatant liquid reserved and used for the determination of cholesterol and such free fatty acid as was not removed by the barium carbonate.

B. *Cholesterol.*

1. *Free cholesterol* (modification of Windaus's [1910] method). 2 cc. of the lecithin-free extract (see note, p. 1090) were evaporated and the fats dissolved in 5 cc. of hot Caminade's [1922] acetone mixture (acetone 73, alcohol 9, water 18 parts).

A slight excess of digitonin in hot Caminade's solution was added and the precipitate allowed to stand for 2 hours at ordinary temperature. The digitonide precipitate was removed and dissolved in 5 cc. of boiling 90 % alcohol. After standing overnight at 18° it was collected in a Gooch crucible, washed with hot water and with a few drops of alcohol and ether, dried at 100° and weighed.

A correction of 0.0002 g. was added to the weight of the precipitate to allow for its solubility in the 5 cc. of alcohol. Factor for cholesterol 0.243.

Notes. (a) The precipitation in Caminade's solution is immediate and quantitative, but in presence of relatively large quantities of fat reprecipitation from alcohol is desirable.

(b) Washing with hot water and the prescribed volume of alcohol and ether does not dissolve a weighable quantity of digitonide [Mueller, 1917].

(c) If the quantity of cholesterol in the extract is very small a larger quantity of extract should be taken since a precipitate weighing 0.0100 g. or more is necessary for accurate determination.

2. *Total cholesterol.* 2 cc. of the extract were evaporated and dissolved in 5 cc. benzene; a quantity of sodium metal, rather more than the weight of total fat, and then 15 cc. of absolute alcohol were added.

The solution was heated under reflux for 8 hours, evaporated just to dryness and the cholesterol extracted repeatedly from the residue with warm light petroleum. The combined extracts were washed with water to remove traces of alkali, evaporated, the residue dissolved in 5 cc. 90 % alcohol, and the cholesterol determined as above. The difference between the total and free cholesterol is the ester cholesterol. The fatty acid was calculated by assuming 40 % to be present in the ester [cf. Mair, 1912].

C. *Total fatty acids.*

These were liberated by heating under reflux 2 cc. of the extract with 1 cc. of 50 % NaOH in 15 cc. alcohol for 4 hours. The alcohol was then removed, the soaps were dissolved in water and transferred to a separator, decomposed with slight excess of 20 % H₂SO₄ and the free fatty acids extracted with light petroleum.

The extract was washed with water until the washings were neutral to methyl orange. The organic acids were then titrated by adding a known volume of N/10 NaOH and titrating the excess with N/10 H₂SO₄ from a microburette in presence of neutralised alcohol using phenolphthalein as indicator. The above method gave satisfactory results with experimental mixtures.

This process was necessary because it is comparatively difficult to hydrolyse kephalin [Levene and West, 1916].

D. Free fatty acids.

2 cc. of the lecithin-free extract were evaporated to dryness, dissolved in 5 cc. light petroleum, washed in a separator until the washings were neutral and then titrated as in C. The amount of fatty acids was calculated assuming a molecular weight of 280 in all cases. The fatty acids removed from the extract by the barium carbonate were added to those calculated from the above titration in order to find the quantity originally present in the extract.

RESULTS OF ANALYSIS OF THIRTY-EIGHT PAIRS OF GLANDS FROM MENTAL
HOSPITAL PATIENTS.

The glands were obtained from autopsies at the Birmingham Mental Hospitals and include 29 male and 9 female cases. It has so far, however, been impracticable to obtain normal human specimens for control. One doubtfully normal specimen has been obtained from a case of veronal suicide and another from a case of accidental death, whose previous history cannot be obtained. For the purpose of comparison the cases were grouped according to age and psychiatric classification.

Total extractives from the glands.

Table I shows the average percentage of extractives in the fresh and dry tissues for the total number of glands.

Table II gives the human series arranged in age groupings.

Table I. *Total fatty extractives.*

Group	No. of cases	% in fresh tissues		% in dry tissues	
		Adrenal	Gonad	Adrenal	Gonad
Whole series	46	11.2	4.3	45.1	23.3
Human series	38	12.4	3.61	47.2	23.0
General paralysis	8	10.9	3.67	39.6	22.6
Dementia praecox	5	8.8	3.7	45.4	23.0
Confusional insanity	6	14.1	3.98	53.4	25.6
Senile dementia	5	19.4	3.6	63.9	28.0
Epileptic insanity	6	13.5	3.43	54.4	20.6
Normal (?) cases	2	12.5	2.86	48.6	23.6
Sheep	3	6.65	3.29	36.4	27.6
Guinea-pigs (mixed glands)	—	19.2	4.25	57.0	27.6
Sow	1	3.63	4.63	15.3	21.1
Cow	2	4.7	—	21.6	—

Table II. *Human cases in age groups.*

Group	No. of cases	% in fresh tissues		% in dry tissues	
		Adrenal	Gonad	Adrenal	Gonad
Under 20 years	4	7.16	2.92	45.7	21.9
20-40 years	11	12.4	3.58	46.2	23.8
40-60 years	16	13.8	3.67	48.5	24.4
Over 60 years	7	17.5	3.31	61.2	21.1

The figures for the analyses of the individual glands show that the gonads, whatsoever their source, contain approximately constant amounts of fat in both dry and fresh tissues; the fatty content of the adrenals however varies considerably.

Amongst the human adrenals the lowest percentages were found in the dementia praecox group and the highest figures in senile dementia. The extractives of the dried adrenal tissue showed definite increase with the age of the subject; this was not due entirely to the glyceride fatty acids since the extractives contained a high proportion of phosphorus-containing lipins.

The figures for the extractives from the adrenals of the cow (21·6 %) and the sow (15·3 %) agree well with those given by Fenger [1916].

Chemical composition of the fatty extractives.

The average percentages of the constituents of the fatty extractives are tabulated for the various groups in Tables III, III A, IV and V.

Table III. *Constituents of extractives %.*

Group	Lecithin	Sphingo- myelin	Total lipin	Free chole- sterol	Chole- sterol ester ¹	Total chole- sterol	Neutral fat	Free fatty acids
Adrenal series	32·9	4·43	37·3	3·72	15·9	13·1	35·9	5·6
Gonad series	37·1	4·48	41·6	7·0	9·55	10·5	35·7	4·2
Human adrenals	33·1	4·55	37·7	3·51	13·7	11·7	38·5	5·1
Human gonads	38·8	4·80	43·6	5·65	10·0	11·6	39·9	4·1
2 normal (?) human adrenals	26·3	9·6	37·6	5·4	21·7	18·5	39·7	5·5
2 normal (?) human testes	37·7	5·43	43·1	8·1	17·3	13·8	34·4	4·9

Table III A. *Constituents of extractives % (animals).*

Group	Lecithin	Sphingo- myelin	Total lipin	Free chole- sterol	Chole- sterol ester ¹	Total chole- sterol	Neutral fat	Free fatty acids
Sheep adrenals	52·6	6·0	58·6	10·8	6·52	14·8	19·3	5·6
Sheep testes	40·0	6·1	46·1	8·6	9·8	14·4	34·1	4·2
Sow adrenals	20·0	2·1	22·5	5·2	2·97	6·92	63·8	3·5
Sow ovary	9·15	1·79	10·9	3·75	21·9	16·9	50·5	3·6
Guinea-pig adrenals	52·7	4·4	57·1	1·56	14·25	10·1	22·9	3·15
Guinea-pig testes	70·4	4·4	74·8	1·85	12·59	9·4	6·5	4·1
Cow adrenals	34·0	5·7	39·7	5·85	0·01	5·9	47·8	6·6

Table IV. *Constituents of extracts % (chief mental groups).*

Mental groups	Lecithin		Sphingomyelin		Total phosphatide		Neutral fat	
	A	G	A	G	A	G	A	G
General paralysis	31·9	33·3	3·36	4·45	35·3	37·8	37·3	41·1
Dementia praecox	27·3	43·3	5·61	6·00	32·9	49·3	33·2	25·3
Epileptic insanity	43·5	34·8	3·83	4·08	47·3	38·9	22·3	43·4
Confusional „	14·3	27·2	3·74	4·1	18·0	31·3	59·3	55·0
Senile dementia	33·3	46·7	4·3	2·6	37·6	49·3	41·6	30·0

Mental groups	Free cholesterol		Cholesterol ester ¹		Total cholesterol	
	A	G	A	G	A	G
General paralysis	3·13	7·25	19·1	10·9	14·9	13·8
Dementia praecox	3·64	4·29	22·8	15·6	17·6	13·9
Epileptic insanity	3·36	7·99	20·7	7·6	16·0	12·4
Confusional „	1·62	2·42	15·3	6·6	10·7	6·4
Senile dementia	3·14	4·58	13·4	6·0	11·2	7·2

¹ Calculated assuming 40 % content of fatty acid [cf. Mair, 1912].

Table V. *Constituents of extracts % (age groups).*

Age group	Lecithin		Sphingomyelin		Total phosphatide		Neutral fat	
	A	G	A	G	A	G	A	G
Under 20 years	31.8	35.8	3.9	3.9	35.7	39.7	55.3	44.2
20-40 years	35.4	42.1	5.9	5.07	41.3	47.2	31.5	33.7
40-60 years	29.0	35.2	3.0	3.2	32.2	38.4	40.1	40.8
Over 60 years	41.3	38.6	3.9	3.9	45.2	42.5	37.0	45.3

Age group	Free cholesterol		Cholesterol ester		Total cholesterol	
	A	G	A	G	A	G
Under 20 years	3.35	3.96	13.3	8.83	11.3	9.3
20-40 years	4.3	5.41	16.3	10.4	16.3	11.2
40-60 years	3.14	5.62	18.6	9.4	14.6	11.3
Over 60 years	3.09	7.05	13.7	4.8	10.6	9.9

The group of confusional insanity shows low values of lecithin and cholesterol, especially free cholesterol. The epileptic group has high figures for the lipins containing phosphorus. The latter observation is opposed to the result obtained by Papea and Eustatziu [1925], who found, in two cases of death after prolonged epilepsy, that the suprarenals showed considerable fibrosis and disappearance of cortex lipins. Our analyses show an average cholesterol value in the adrenals of epileptics and in no individual case was the amount much less than the average for the total glands.

The average figure for phosphatide content of human testes was 43.6 % of the extract (1.58 % of the dry tissue). This is somewhat higher than that obtained by Mann in cases of mental disorder (half of which, however, had carcinoma).

One of the specimens, the testes of a senile dement (A. E. K.), contained a very high proportion of cholesterol ester (29 %). These glands weighed only 2.26 g. and were very fibrous with hard, thick capsules.

Guinea-pig adrenals were found to contain a very high proportion of phosphorus-containing lipins (57 %). Similar results are quoted by Elliott and Tuckett [1906].

One specimen of adrenal from a cow contained no cholesterol esters, although a moderate amount of free cholesterol was present. A similar absence of combined cholesterol has been reported by Sorg and Jaffé [1924]. The reverse condition, *i.e.* absence of free cholesterol and presence of ester cholesterol, was observed by Rosenheim and Tebb [1909] in the adrenals of cattle.

Relative proportions of phosphatide and cholesterol.

In the investigation of a possible relationship between the amounts of total phosphatide and total cholesterol (free cholesterol and cholesterol from the esters) an estimate was made of the relative proportion in which these constituents were present in each gland.

It was found that in half the adrenals the relative proportion of cholesterol to phosphatide was between 1 : 1.5 and 1 : 3.0, the remainder differing widely from these figures; in about one-third of the gonads the proportions were

between 1 : 2·0 and 1 : 5·0. The gonads showed a progressive decrease in the lipin-cholesterol proportion when considered in relation to the age grouping (Table VI).

It is interesting to note that in the group of confusional insanity the adrenals had a remarkably low proportion of phosphatide to cholesterol, while the lipin-cholesterol proportion in the gonads remained high. There is thus a wide variation in this class which exceeds any other grouping. The low lipin-cholesterol proportion in the adrenal was due to the low percentage of phosphatide and suggests that there is a relation between the disappearance of this constituent and that process which has been shown to occur in toxic conditions, existing over long periods, by Elliott and by Mott. These workers found that chronic infection results in marked diminution of cortex lipids.

The main object for which this work was undertaken was to determine whether chemical evidence supported the view of the function of the adrenal in elaborating the cholesterol and phosphatide for the developing and functioning gonads. It is apparent that if these constituents are not changed or further elaborated the ratio obtained by dividing the proportions of phosphatide to cholesterol in the adrenal by the proportion in the gonads would be unity. These ratios have been accordingly calculated and are given in Table VI. It was found that many of the ratios varied widely from the

Table VI. *Relative proportions of cholesterol and phosphatide.*

Average for	Whole series	General paralysis	Dementia praecox	Epileptic insanity	Confus. insanity	Senile dementia	Under 20 years	20-40 years	40-60 years	Over 60 years
Adrenal	2·8	2·4	1·95	3·0	1·7	3·4	3·1	2·5	2·2	4·3
Gonad	4·0	2·7	3·55	3·1	4·8	3·7	4·25	4·2	3·4	4·2
Ratio	0·70	0·89	0·55	0·97	0·35	0·92	0·73	0·60	0·65	1·0

expected figure and therefore this view is not supported by chemical analyses of the glands. Even allowing the wide margin of 0·5 to 1·5 only half the total figures fall within these limits. Of considerable interest, however, is the wide divergence from the normal of the group of confusional insanity. This has the low ratio of 0·35 due to the low proportion of phosphatide in the adrenal which, as stated previously, probably results from the toxic condition associated with these cases.

SUMMARY.

Analyses of the total alcohol-ether soluble extractives of the adrenals and gonads of 38 patients in mental hospitals have been carried out by special methods.

The percentages of total fatty acid, lecithin, sphingomyelin, free and ester cholesterol and free fatty acid have been determined and the average results for various mental and age groups are given. The percentage amount of fatty extractives from the gonads was found to show much less variation than that from the adrenals. The lowest adrenal content was found in dementia praecox and the highest in senile dementia.

Low values of phosphatide were obtained in the adrenals of the confusional group, and high figures were found in the adrenals of epileptics.

The relative proportions of the total cholesterol to the total phosphatide varied greatly in the cases examined, and the ratio of these proportions in the pairs of glands differed from unity in a marked degree so that the evidence from quantitative chemical analysis of the fatty constituents does not support the view that the lipoids of the gonads are elaborated by and transported directly to these glands from the adrenal cortex.

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