FATTY ACID METABOLISM IN THE LIVER[1]. I. A METHOD OF FATTY-ACID EXTRACTION.

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THE object[2] of this work has been to evolve a method that (i) is consistent with itself to 1 %, (ii) yields more fatty acid than the Rosenfeld method, (iii) does not oxidise the fatty acid in the process of drying and extraction, and (iv) gives a pure product. But in addition an interesting set of observations that bear upon Leathes' theory of the function of the liver in fatty acid metabolism has been made in the course of the investigation and will be dealt with briefly in the concluding paragraph.

It is obvious to anyone who has followed the estimation of fatty acids in organic tissues that until the papers published by Glikin[3], Leathes[4] and Kumagawa and Suto[5], researchers who advocated various methods were not certain (i) that their method extracted the total fats (or fatty acids[6]) in the tissue; (ii) that it did not destroy the fats (or fatty acids[6]) in the processes of extraction and drying; and (iii) that the extract was pure fat (fatty acids or soaps[6]).

Inferential arguments alone can meet the first two uncertainties. We can never have an absolute certainty as long as we are estimating substances in organic tissues. The third, Leathes and Kumagawa and Suto, who show that a Rosenfeld ether extract of liver or muscle may contain 40 % impurities, have proved to be only too well founded.

By "fatty acid" is meant throughout this paper the non-volatile higher fatty acids which are soluble in petrol ether (B.P. 40—60°C.)

[1] Part of the expenses of this research has been defrayed by a grant from the Royal Society.

[2] The method has been already sketched in a previous Paper. This *Journal*, XXXVIII. p. 291. 1909.

[3] *Pflüger's Arch.* XCV. p. 107. 1903.

[4] *Proc. Physiol. Soc.* p. 1. 1904. (This *Journal*, XXXI.)

[5] *Biochem. Zeitschr.* VIII. p. 212. 1908.

[6] The methods of Liebermann and Kumagawa estimate fatty acids—the former as soaps.

and form potassium salts soluble in 50 % alcohol. It is these and these only that have been estimated.

I. THE METHOD ADOPTED.

(*a*) *General sketch of the Method.*

The method is substantially as follows:

The organ (liver) is cleared from visible fat and connective tissue and minced. A weighed portion is dissolved in 60 % caustic potash. The fatty acids are precipitated by mineral acid and extracted with ordinary ether. The resulting extract (consisting of acid hæmatin, fatty acids, cholesterol and other lipoids) is dried, dissolved in petrol ether and filtered. The resulting solution of lipoids is separated into soaps and unsaponifiable substances by means of a saturated solution of caustic potash in absolute alcohol diluted with distilled water to twice its volume. The soaps are separated, the fatty acid reprecipitated and extracted with ether.

As originally described[1] the *crude* fatty acids were dried *in vacuo* at 100° C. and weighed. The unsaponifiable substances were then extracted, dried *in vacuo* at 100° C. and weighed. The difference between the two weights was taken as representing fatty acids. This procedure was soon discarded as requiring two sets of weighings, and yielding no fatty acids for the iodine value estimation without a further weighing.

By shaking the solution of lipoids in petrol ether with alcoholic potash, the potassium salts of the fatty acids pass into the 50 % alcohol. They can then be run off, the fatty acids reprecipitated by mineral acid and extracted with petrol ether. This represents a saving of 6 to 8 hours, and is now always adopted.

The solution of purified fatty acids is then filtered, dried *in vacuo* at 100° C. and weighed. The iodine value estimations are carried out on the fatty acids thus obtained.

The various modifications of the method are detailed below as they became necessary, and an account of the method as now carried out with practical details is given at the end of the paper.

(*b*) *The uselessness of petrol ether as original extraction fluid.*

An attempt was made to substitute petrol ether for ordinary ether in the first extraction. It was thought that this would avoid the extraction of acid hæmatin, lactic acid and the other non-lipoid substances unfortunately soluble in ether, and save the subsequent

[1] This *Journal*, *loc. cit.*

purification of the ether extract. But petrol ether, in the presence of the hydrolytic products of the liver, behaves in so fickle a way as to make its use impossible. Not only did it form intractable emulsions, but the results were, on the whole, unreliable. Sometimes good results were obtained[1], but normally there were big errors, as the following figures show.

EXP. 1. Bullock's liver, minced, and portions weighed out into Schott and Gen flasks. As much 60 % KOH solution as gms. liver added, and the whole boiled 3 hrs. on a water-bath. Subsequent treatment as above.

Weight of liver	Fatty acids obtained	% of fresh liver	Iodine value
23·20 gms.	1·4808 gms.	6·38	107
31·25	1·7282	5·53	104
27·08	1·7662	6·52	111
25·82	1·7678	6·85	116

Two other experiments yielded results as bad. The fatty acids, however, when examined showed no trace of "unsaponifiable substance,"[2] so that the method, if it gives irregular results, yields a pure substance. These irregularities were immediately overcome on reverting to the original method.

EXP. 2. Lamb's liver, minced, mixed and portions weighed out into Schott and Gen flasks. Boiled with 60 % KOH as usual, 3 hours. The resultant solutions diluted somewhat, cooled and made up to 250 c.c. Aliquot parts taken at once (60 c.c.) and extracted as described above.

	Weight of liver	Fatty acid	% of fresh liver	Iodine value
(*a*)	38·35 gms.	0·6772 gms.	7·36	115
(*b*)	42·10	0·7492	7·42	119
			Error ±0·41 %	1·7 %

Two days later two more aliquot parts were pipetted off (50 c.c.). The soaps had risen to the surface and it was difficult to mix the solution thoroughly.

	Fatty acid	% of fresh tissue	Iodine value
(*a*)	0·6373 gms.	8·32	117
(*b*)	0·7233	8·58	120

All difficulties disappeared on the adoption of ordinary ether[3], the troublesome emulsions met with when using petrol ether were almost absent, the fatty acids were less pigmented, and the results of the first aliquot parts taken showed a good agreement: error ± 0·41 %. The

[1] See this *Journal*, XXXVIII. p. 291. 1909.

[2] See Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats and Waxes.* (4th Ed.). vol. I. p. 363.

[3] Commercial ether sulph. meth. purified according to the method described by Kumagawa (*loc. cit.*).

higher results in the estimations of the liver solutions allowed to stand two days were put down at the time to the separating out of the soaps that was observed to take place. As a matter of fact that the first estimates agreed so well was a lucky accident, as the next paragraph will show.

(*c*) *The impossibility of using aliquot parts of the caustic potash solution.*

An attempt to save time in weighing portions of the organ for parallel estimations, by taking aliquot parts of a large quantity of caustic potash solution of liver, led to disaster. Sometimes the experiment succeeded, but only when the fractions taken were a large part of the whole (*e.g.* $\frac{1}{2}$) or were pipetted off immediately after solution. The soaps separate out so rapidly that the experiments often fail.

Exp. 3. Bullock's liver, minced. Two portions weighed out into Schott and Gen flasks and dissolved 3 hours on a water-bath with 60 % caustic potash. Each portion on cooling was made up to 200 c.c., 100 c.c. were taken.

	Weight of liver		Fatty acid		% of fresh tissue	Iodine value
(*a*)	30·45 gms.	(i)	0·4947 gms.		3·25	137
		(ii)	0·4894		3·21	136
(*b*)	24·08	(i)	0·3929		3·26	136
				Mean	3·24	136·3
				Error	±0·67 %	± 0·3 %

Small glass stoppered flasks were used in this experiment for the final distillation in CO_2 and for the weighing.

The experiment must be counted a success in spite of the use of aliquot parts. The error is ± 0·67 %.

Exp. 4. Bullock's liver treated as above, the solutions made up to 250 c.c. and 50 c.c. taken for each estimation. Weights of liver (*a*) 52·08 gms., (*b*) 45·47 gms.

	Fatty acid		% of fresh tissue	Iodine value
(*a*)	0·5936 gms.		5·69	108
	0·5914		5·67	112
	0·5911		5·67	—
	—		—	109
		Mean	5·677	109·7
(*b*)	0·5090		5·60	104
	0·5201		5·72	109
	0·5071		5·58	103
(100 c.c.)	1·0402		5·72	110
Total	2·5764	Mean	5·66	108·7
		Error on means	±0·15 %	± 0·46 %

It will be seen that whereas in the first set of estimations the taking of aliquot parts does not influence the accuracy of the results, in the second the error introduced is great. The means of the two sets of results agree excellently.

One other experiment may be briefly mentioned.

EXP. 5. 126·7 gms. calf's liver treated as above, solutions made up to 500 c.c. and 50 c.c. used for each estimation.

Fatty acid	Iodine value	Fatty acid	Iodine value
0·2850 gms.	140	0·2954 gms.	131
0·2669	135	0·2892	141
0·3543	134	0·2992	128
0·2962	140		

After this result (which gives some evidence that the soaps fractionate out) attempts to use aliquot parts of the liver solution were finally abandoned.

(*d*) *The influence of the time of solution.*

An early experiment suggested that the time the liver was heated with caustic potash materially affected the resulting amount of fatty acid obtained.

EXP. 6. Bullock's liver treated as above. Two of the portions, (*a*) and (*b*), dissolved with great difficulty owing to the quantities taken being small for the litre flasks used. Solution was completed only shortly before the end of the three hours. The other two dissolved rapidly and completely.

	Weight of liver	Fatty acid	% of fresh tissue	Iodine value
(*a*)	11·17 gms.	0·3500 gms.	3·13	143
(*b*)	12·90	0·4052	3·14	—
(*c*)	17·40	0·5593	3·21	146
(*d*)	16·93	0·5402	3·19	144
		Error of (*c*) & (*d*)	±0·32 %	±0·7 %

The first pair agree with each other, and the second also as closely as is necessary in estimation, but between the means of these pairs the disagreement is hopeless. It will be seen that the two portions that dissolved slowly, yield less fatty acid than those that dissolved rapidly, but as the iodine value is the same, approximately, this cannot be due to oxidation but rather to defective hydrolysis. It may not be out of place here to insist on the necessity of rapid solution in caustic potash, since several imperfect results have been traced to insufficient care in shaking the flasks during the early stages of dissolution.

Further experiments were then made to determine the length of time of solution that yields the largest amount of fatty acid.

EXP. 7. Bullock's liver minced and weighed off into pressure bottles (about 150 c.c. capacity); to these the requisite quantities of 60 p.c. KOH were added. The bottles were then exhausted of air, the corks securely tied-in and immersed for varying times, with frequent shakings, in a water-bath. The rest of the estimation proceeded as usual.

	Weight of liver	Time of solution	Fatty acid	% of fresh tissue	Iodine value
(*a*)	9·75 gms.	½ hr.	0·4870 gms.	5·00	124
(*b*)	13·73	1½ hrs.	0·7118	5·18	128
(*c*)	15·08	3	0·7765[1]	5·15	125
(*d*)	15·10	4½	0·7844	5·19	128
			Mean of (*b*), (*c*) & (*d*)	5·177 Mean	126·3
			Error	±0·32 % Error	±1·41 %

It will be seen that half an hour on the water-bath is insufficient, whereas from 1½ hours to 4½ hours there is no change in the recovered fatty acids. This observation has a twofold value: (i) the time of solution may be reduced from 3 hours to 1½ hours—a considerable saving, and (ii) it shows that the fatty acids are not attacked by the strong caustic potash used.

This important observation was confirmed by the following experiment.

EXP. 8. Rabbit, weight 3600 gms., without food 18 hours, killed by rapid bleeding. Liver, weight 84·65 gms., minced as usual, portions transferred to pressure bottles and treated as in EXP. 7.

Weight of liver	Time of solution	Fatty acids	% of fresh tissue	Iodine value
9·50 gms.	1½ hrs.	0·3798 gms.	4·00	104
10·29		0·4042	3·93	99
12·88	4½	0·5111	3·97	—
10·61		0·4215	3·97	104
		Mean	3·97	102·3
		Error	±0·63 %	± 2·3 %

(*e*) *The effect of absence of oxygen during solution.*

The rapidity with which the fatty acids of the liver oxidise has been emphasised by Hartley[2], and it has been shown that in drying fresh tissue in a hot air oven considerable oxidation takes place[3]. It was therefore necessary to see if the carrying out of the solution in caustic

[1] This weight should be somewhat higher, for a small fly drowning itself in the final petrol extract, necessitated another filtration.

[2] This *Journal*, XXXVI. p. 17, 1907, and XXXVIII. p. 353. 1909.

[3] This *Journal*, XXXVIII. p. 291. 1909.

potash in the presence of air caused a diminution of the yield of fatty acid. As a matter of fact it could be argued that during solution there is little air present, the gases in contact with the fluid being mainly water-vapour. In three experiments done to ascertain the amount of oxidation, it was found to be *nil.*

EXP. 9. Bullock's liver. Two portions treated as in EXP. 7, and two others similarly except that the pressure bottles were left uncorked. Time of solution 3 hours in every case.

Weight of liver	Fatty acids		% of fresh tissue	Iodine value	Remarks
20·57 gms.	—		—	98·2	vacuum
12·10	1·8447 gms.		15·25	94·9	
13·60	2·1082		15·50	99·6	air
12·90	1·9618		15·21	96·6	
		Mean	15·32	97·3	
		Error	± 0·84 %	± 2·1 %	

This not altogether satisfactory experiment (in view of the large amounts of fat present and the low iodine values) was repeated on another sample of bullock's liver.

EXP. 10. Bullock's liver treated as in EXP. 9.

Weight of liver	Fatty acids		% of fresh tissue	Iodine value	Remarks
10·24 gms.	1·6543 gms.		16·15	89·7	vacuum
9·86	1·5741		15·96	91·4	
10·70	1·6974		15·86	89·8	air
		Mean	15·99	90·3	
		Error	± 0·75 %	± 0·86 %	

As in Exp. 9 there seems to be no oxidation taking place, but the results are slightly in favour of using a vacuum. The experiment was repeated with the liver of a cat that had been fed for the previous four days on herrings. As Leathes[1] has shown such feeding always raises the iodine values of the fatty acids of the liver. This course was adopted to give the greatest possible chance for oxidation to take place.

EXP. 11. Cat's liver treated as in EXP. 9.

Weight of liver	Fatty acid	% of fresh tissue		Iodine value	Remarks
13·73 gms.	2·1364 gms.	15·52	15·45	156	vacuum
9·77	1·5033	15·38		150	
9·90	1·4889	15·04	15·19	157	air
10·31	1·5812	15·34		154	

Analysis of a sample that had been allowed to autolyse at 37 ° C. *in vacuo* two days and was dissolved in KOH out of contact with air, yielded 15·52 per cent. fatty acid, with an iodine value 154. Mean of iodine values 154·2. Error ± 1·6 p.c.

[1] *Lancet*, Feb. 27th, 1909.

It is therefore clear that, even with highly oxidisable fatty acids in the liver, the oxidation that takes place during solution is negligible. There is a slight advantage however in hydrolysing *in vacuo*, but at present I have no explanation to offer of this, unless it be that hydrolysis is often increased and hastened under pressure. The large evolution of ammonia from the liver during solution must raise the pressure within the pressure bottle. To secure this advantage the bottles are now always stoppered, and to prevent any possible oxidation under these circumstances the air is abstracted.

(*f*) *The effect of a preliminary autolysis of the tissue.*

It was thought that any fatty acid linkage not broken down by caustic potash would conceivably succumb to the autolytic ferments present. Consequently several autolysis experiments were carried out. At the same time the possible synthesis of fats from carbohydrate (?) and the "desaturation" of fatty acids in anærobic and antiseptic conditions were studied[1].

EXP. 12. A preliminary experiment was made on the liver of a cock that had been without food from the previous evening. One portion of the minced liver was analysed at once as in EXP. 7; the rest after a two days' autolysis in the presence of toluol and air at 37° C.

Weight of liver	Fatty acid	% of fresh tissue	Iodine value	Remarks
9·1 gms.	0·2751 gms.	3·02	104	control
6·1	0·1849	3·03	113	autolysis
	Mean	3·025	108·5	
	Error	±0·16 %	± 3·2 %	

The estimations agree excellently as regards percentage fatty acid, but the iodine values disagree. This, though not surprising in view of the small amounts of fatty acid at disposal for estimation was considered worthy of further attention (see below).

The later experiments were made with livers that autolysed *in vacuo*.

EXP. 11 *a*, for protocol of control see EXP. 11 above. The sample for autolysis was weighed off into a pressure bottle, toluol was added and the bottle exhausted of air. The liver autolysed at 37° C. for two days. Abundant evidence of autolysis was shown, but the liver showed no signs of putrescence.

Weight of liver	Fatty acid	% of fresh tissue	Iodine value	Remarks
14·41 gms.	2·2355 gms.	15·52	154	autolysis
—	—	15·45	153	mean of two controls
	Mean	15·485	153·5	
	Error	± 0·23 %	± 0·33 %	

[1] *Lancet*, Feb. 27th, 1909.

There is obviously no estimable difference between the autolysed liver and the controls.

Exp. 13. Livers of two hungry rabbits of 2350 gms. and 1250 gms. weight respectively. The animals had been without food for 20 hours. Weights of livers 61·60 gms. and 30·15 gms. respectively. These were minced and mixed, two samples weighed out for immediate analysis, and two allowed to autolyse in presence of toluol and absence of air two days at 37° C. Analysis as usual. A third sample for autolysis was not weighed and the autolysis took place in air.

	Weight of liver	Fatty acid	% of fresh tissue	Iodine value	Remarks
(*a*)	9·90 gms.	0·2942 gms.	2·97	107	control
(*b*)	10·42	0·3125	3·00	104	
(*c*)	11·14	0·3325	2·99	106	anærobic
(*d*)	10·80	0·3283	3·04	108	autolysis
(*e*)	—	0·3806	—	101	semiærobic autolysis
		Mean of (*a*) to (*d*)	3·00	106·3	
		Error	±0·85 %	± 1·4 %	

There is then no evidence in these experiments that an antiseptic anærobic autolysis produces a greater yield of fatty acid than a direct solution in caustic potash. Evidence will be given below that autolysis does not influence the *nature* of the fatty acid as evidenced by its iodine value. It is almost inconceivable that no alteration in the nature of the fatty acids in an autolysing tissue would be compatible with a change in the amount present, and thus the above experiments receive an indirect confirmation.

(*g*) *Comparison with Rosenfeld's method.*

That Rosenfeld's method of fat estimation yields a product that is not pure fat has been pointed out again and again. It was thought necessary however to compare the method adopted with Rosenfeld's and to hydrolyse the ether extract obtained by the latter method and purify the fatty acids from it for direct comparison.

Exp. 14. Bullock's liver minced, three samples taken for control, and one large sample for a Rosenfeld estimation. The former were treated in the usual way, the latter dried to constant weight *in vacuo* at 100° C. It was then thoroughly ground, re-dried, and weighed portions extracted twice with absolute alcohol and chloroform. The extractions were pushed somewhat to extreme in order that all extractible substances should be removed. The extracts were evaporated in a stream of CO_2 to dryness, taken up in ether and filtered into a weighed bottle, again evaporated to dryness in CO_2 and farther dried *in vacuo* at 100° C. till of constant weight. This crude ether extract (which is solid at 100° C. !) was then hydrolysed with 50 c.c. strong alcoholic potash for three hours. The resultant fluid was then treated exactly as if it were a solution of fresh liver in caustic potash and the pure fatty acids prepared from it.

Results. (i) The method under investigation.

Weight of liver	Fatty acids		% of fresh tissue	Iodine value
15·90 gms.	0·5611 gms.		3·53	148
18·67	0·6595		3·53	148
13·06	0·4554		3·49	148
		Mean	3·517	148
		Error	±0·54 %	nil.

(ii) Rosenfeld.

66·53 gms. fresh tissue = 19·60 gms. dry. 1 gm. dry = 3·394 gms. fresh tissue.

Weight of dry liver	Equivalent in fresh tissue	Crude extract	% of fresh tissue
7·3825 gms.	25·06 gms.	1·4875 gms.	5·94
4·8203	16·36	1·0226	6·11
6·1099	20·73	1·2195	5·88

Pure fatty acids	% of fresh tissue	Iodine value of pure fatty acids
—	—	134
0·5438 gms.	3·32	134
0·6495	3·13	138
Mean	3·225	135·3
Error	±2·9 %	± 1·4 %

The method is therefore not only less tedious (four parallel estimations can be made in 2½ days), but produces more fatty acid than Rosenfeld's (in the relation of 100 : 92) and does not oxidise the fatty acid in the process. The Rosenfeld estimations, though not agreeing[1] very well, are favourable to the method: the tissue was dried *in vacuo*, thus avoiding oxidation.

(*h*) *Detailed description of the method.*

After the removal of visible fat and connective tissue the organ is minced, and thoroughly mixed. Portions (10—15 gms.) are then introduced through a funnel into weighed pressure bottles of about 150 c.c. cubic content. The bottles are then weighed again and to each is added as many c.c. 60 p.c. caustic potash as there are grammes of fresh tissue. The bottles are then stoppered with rubber-stoppers and exhausted of air by means of a water-pump. Next they are immersed in a boiling water-bath for 1½ hours, during the earlier part of which time they must be frequently shaken. Neglect of this precaution leads to anomalous results. The caustic potash solution is transferred hot to a conical separating funnel of 250 c.c. capacity, the stoppers of which have previously been moistened with glycerine. The pressure bottle is rinsed thoroughly into the separating funnel with warm water, and the combined solutions cooled under the tap. 20 c.c. strong hydrochloric acid are added slowly and a copious precipitate results. The acid solution is cooled under the tap and shaken for 30 secs. with 50 c.c. purified ethyl ether. A separation occurs very rapidly. The acid is run off into a beaker until the precipitate which rises to the junction of ether and acid begins to leave the funnel. The ethereal solution of fatty

[1] Due, probably, to imperfect hydrolysis of the Rosenfeld extract. This difficulty in hydrolysis is not surprising in view of the large amount of waxy impurities in the Rosenfeld extract.

acid etc., is then siphoned off into a CO_2 flask and the funnel rinsed twice with 10 c.c. ether. The rinsings are siphoned off in the same way.

The precipitate is dissolved in a few drops of strong caustic potash and shaken with 50 c.c. ether for 30 secs. Before the emulsion has time to settle the acid solution from the beaker is added to the separating funnel and the mixture shaken for a further 30 secs. If separation does not take place at once the funnel is placed for a few minutes in an incubator at 37° C. When separation is complete, the acid solution, but not the precipitate, is run off, and the ethereal solution siphoned off into the original CO_2 flask. The funnel is rinsed as before.

The solution of the precipitate is repeated, but in extraction instead of 50 c.c. ether, 25 c.c. are used. Otherwise the process is as before.

The united ether extracts are then evaporated nearly to dryness in a current of CO_2. Absolute dryness results in pigmentation of the fatty acids. The extract containing a very small amount of ether is left to stand in an atmosphere of CO_2, preferably overnight, by which time the ether will have evaporated and left a brown crystalline mass.

This is dissolved in petrol ether[1] (about 50 c.c.) and the resultant mixture is filtered through an asbestos *fibre* filter previously described[2] into a separating funnel of 250 c.c. capacity. The CO_2 flask is thoroughly rinsed with petrol ether and the washings filtered into the same funnel. The filter is similarly thoroughly rinsed, especially the lower end.

50 c.c. of a mixture of equal parts of strong alcoholic potash (made with absolute alcohol) and water, together with a few drops of phenolphthalein are added and the funnel shaken 30 secs. On separation (in the decision of which the phenolphthalein aids) the subnatant fluid containing the potassium salts is run off into a graduated cylinder of 250 c.c. capacity. The washing of the petrol ether solution is repeated with 50 c.c. and 25 c.c. of the potash in 50 p.c. alcohol.

To the united alcoholic solutions of the potassium salts of the fatty acids, 5 c.c. of concentrated HCl are added to precipitate the fatty acids afresh. These are extracted three times with petrol ether (twice with 50 c.c. and once with 25 c.c.) and the ether on separation is siphoned off into a CO_2 flask each time. Between each extraction the funnel is rinsed twice with 10 c.c. petrol ether and the rinsings siphoned off into the same CO_2 flask.

The united extracts are evaporated in a stream of CO_2 till the fluid measures about 20 c.c. This condensed solution is filtered through asbestos fibre into a clean weighed glass flask (weighing from 20—35 g.) fitted with a ground-in stopper with short entrance and exit tubes. The CO_2 flask and filter are carefully washed with petrol ether into the small flask. The ether is distilled off in a stream of CO_2, and the dried fatty acids further dried, till of constant weight, in a vacuum oven[3] at 100° C. This takes about six hours.

After the fatty acids are dried and weighed, they are dissolved in carbon tetrachloride, transferred quantitatively to a Sendtner flask and their iodine value estimated by Wijs' method.

II. The effect of antiseptic autolysis on the iodine value.

As was shown in the three experiments quoted above (11*a*, 12 and 13) no change takes place in the amount of fatty acid in the liver on

[1] The petrol ether used is manufactured by Messrs Carless, Capel and Leonard, Hackney Wick, and distils between 40° C. and 60° C.

[2] This *Journal*, xxxviii. p. 291. Asbestos fibre is far preferable to wool.

[3] The vacuum oven is one used in the beet sugar industry and is listed by Messrs Gallenkamp.

antiseptic autolysis and no reliable change in the iodine value. Two of the autolyses were anærobic and two semiærobic. By the latter is meant that though the exterior of the liver pulp was in contact with the air, the interior is probably highly reducing[1]. In Leathes' experiments[2] on autolysis of the liver with increase of the fatty acids resulting, the autolyses were ærobic; Siegert's[3] on the other hand were only semiærobic. This simple difference in the method of experimentation may very well account for the differences in their results. Leathes obtained an increase of fatty acid, Siegert an entirely negative result.

The problem to be solved in the following experiments was not so much as to whether the fatty acids were increased in autolysis but as to the change in the nature of the fatty acids, if any. Leathes, in the remarkable lecture already quoted, has suggested that the function of the liver in fat metabolism is to desaturate the fatty acid radicles brought to it from the fat dépôts, for consumption in the tissues where large amounts of energy are needed. It was thought that indications of this process of desaturation might be observed in autolysing livers. Above, it has already been said that, though theoretically possible, it is hardly conceivable that an increase of fatty acids can take place without a concomitant change of the iodine value. Three experiments have been given above to show that the percentage of fatty acids does not alter in anærobic and semiærobic autolysis, and in only one of these did a change (and a doubtful change at that) take place in the iodine value.

In one experiment (Exp. 8*a*) on the liver of a hungry rabbit, a rise in the iodine value with a concomitant decrease in the percentage did take place. This result I have not yet been able to confirm.

Exp. 8*a*. For protocols of controls see Exp. 8. One portion of the minced liver was weighed out into a pressure bottle and allowed to autolyse for two days in presence of toluol and absence of air at a temperature of 37° C.

Weight of liver	Fatty acid	% of fresh tissue	Iodine value	Remarks
11·28 gms.	0·4377 gms.	3·88	128	Autolysis
—	—	3·97	102	Mean of four controls

Exp. 15. Two rabbits without food 45 hours. Original body weights 2800 g. and 3100 g. Liver weights 56·60 g. and 69·00 g. Five portions of the minced and mixed livers taken, two for autolysis and the rest for controls.

	Controls	Two days' autolysis
Iodine value of fatty acids	114, 116, 111, (mean 113·7)	113, 117 (mean 115)

[1] See Weinland, *Zeit. f. Biol.* XLVIII. p. 87. 1906.

[2] *Schmiedeberg's Arch.* Supp. 1908.

[3] *Hofmeister's Beitr.* I. p. 114. 1902.

Exp. 16. Livers of three hedgehogs minced and mixed. Two portions allowed to autolyse under toluol at 37° C. and in absence of air six days. Three portions used as controls.

	Controls	Autolysis
Iodine value of fatty acids	132, 139, 135 (mean 135·3)	139, 139

From these experiments and the ones quoted above I (*f*) we can say that a desaturation of the fatty acids during antiseptic anærobic autolysis, even where most to be expected—in the livers of hungry animals—is a rare phenomenon. It is impossible however to affirm its constant absence in view of the single positive result, and all that we can say is that it takes place in no large percentage of experiments. The results so far are 8 to 1 against with one doubtful desaturation.

Summary.

(1) A method of fatty acid estimation requiring only 10 gm. of liver has been worked out and the method described in detail (v. p. 131).

(2) The method is consistent with itself both in the percentage of the fatty acid yielded and in the iodine values of the fatty acid. The error in the former ranges from ±0·15 per cent. to ±0·85 per cent., the average error being ±0·51 per cent. The error in the iodine values ranges from *nil* (three parallel estimations agreeing identically) to ±3·2 per cent., with an average error of ±1·54 per cent.

(3) The method yields more fatty acids than Rosenfeld's.

(4) In antiseptic and anærobic autolysis of the liver little evidence has been discovered of an alteration in the fatty acids either in amount or in the direction of desaturation.