CLXXVII. THE EFFECTS OF PANCREATIC EXTRACTS ON FAT DEPOSITION IN THE DIETARY FATTY LIVER

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ALLAN *et al.* [1924] reported that depancreatized dogs receiving adequate amounts of insulin and maintained on a diet of lean meat, sucrose and bone ash did not survive for periods of more than a few months. They also observed that the symptoms of failure of liver function due to fat infiltration of the liver found in such animals, could be prevented by adding raw pancreas to the diet. They suggested, therefore, that the pancreas might possibly produce an internal secretion necessary for the physiological integrity of the liver. The subsequent discoveries of Hershey [1930] that lecithin could successfully replace raw pancreas in the diet of depancreatized dogs, and of Best & Huntsman [1932] that choline and betaine could prevent and cure fatty infiltration in the livers of rats fed on high fat diets have been extended in many directions. So far as we are aware, however, no proof has yet been advanced that the active agent of the pancreas is, in fact, choline, and particular interest therefore attaches to the claims of Dragstedt and his colleagues [Dragstedt *et al.* 1936, 1, 2] that raw pancreas contains an active substance other than choline and that this substance, to which they give the name "lipocaic" may be concentrated in dilute alcoholic extracts of the tissue.

The work described in this paper was undertaken in an attempt to extend these findings to the dietary fatty liver of the rat, which lends itself to more accurate experimentation than that of the depancreatized dog.

While these experiments were in progress, other workers reported the results of similar studies [Chaikoff & Kaplan, 1937; Kaplan & Chaikoff, 1937; MacKay, 1937; Shapiro & Wertheimer, 1937; Aylward & Holt, 1937; MacKay & Barnes, 1938; Best & Ridout, 1938]. Some of these results throw doubt on the existence of a pancreatic factor other than choline influencing fat deposition in the liver, while in some cases the conclusions drawn appear to us to be based on inadequate data. These points are discussed later in this paper.

The general plan of the experiments has been to feed control groups of rats on diets which cause fatty livers. At the same time other groups have received the same diet with the addition of varying amounts either of pancreatic extract or of choline, in order to assess in terms of choline the activity of pancreatic extract in preventing fat deposition. It is difficult to strike the correct range of choline dosages in attempting to assay an unknown substance and many more groups of animals were used than are recorded in the tables which follow. Tables II-V contain, therefore, only the results of the 42 groups which are relevant to the discussion, either because they provide the actual results on which the effect of the pancreatic extract has been assessed, or alternatively because it is important in forming a decision as to the activity of the pancreatic

extracts, that the effects of increasing amounts of choline should be recorded for comparison, in order that the accuracy of the assay may be suitably appraised.

Because of the differences of opinion evident from the publications referred to above, it appeared to us necessary to obtain a considerable body of evidence. Accordingly six experiments were carried out, three on the "cholesterol", and three on the "fat" fatty liver.

EXPERIMENTAL

Preparation of the extracts

Fresh ox pancreas obtained from the slaughter house was freed from extraneous connective tissue and fat.

Dragstedt *et al.* [1936, 2] extracted pancreas with saline and with alcohol of various dilutions and found that saline or 40 % alcohol gave more active extracts than 70 % alcohol. In the experiments now reported the method of extraction used has followed closely that of these workers except in two particulars: (a) in the initial extraction of each batch alcohol was added so that, allowing for the water content of the pancreas, the final concentration was 40 %, all the succeeding extractions being carried out with 40 % alcohol save in Exp. 2; (b) instead of evaporating the extracts in open dishes and extracting the residual solid with ether, alcohol and water were removed *in vacuo* and extraction carried out by shaking with ether in a funnel before the material became pasty. In all the experiments the extracts were made up to a definite volume, and stored in the refrigerator: aliquots providing sufficient of the extracts for incorporation in 3-day portions of diet for all groups receiving pancreatic extract were withdrawn into test tubes, pasteurized, sealed and stored in the refrigerator for use as required.

Exp. 1. 1.2 kg. of minced pancreas were left overnight with 2.4 l. of 60 % alcohol, strained through muslin and twice re-extracted with 40 % alcohol (2.4, 2.4 l.); the residue was treated with ether (2.5, 2.0, 2.0 l.). The first "ether" extract which contained much alcohol and water was added to the 40 % alcohol extracts and the whole evaporated *in vacuo* at 60°. Fat was removed from the concentrate by shaking with the second ethereal extract of the protein residue and four times subsequently with fresh ether (1 l.); alcohol and ether were then removed from the aqueous suspension *in vacuo* and the volume made up to 1.2 l. with water.

Exp. 2. 5 kg. minced pancreas were treated three times with 40 % alcohol as in Exp. 1: the protein residue was then extracted with 95 % alcohol (2, 3 l.), the extracts evaporated *in vacuo* and added to the previous extracts. Much of the fat thus added to the 40 % alcohol extracts was removed by ether, but the high choline content (Table I) indicates that some phosphatide was probably left in the aqueous layer.

Exp. 3. 1.2 kg. of minced tissue worked up as in Exp. 1.

Exp. 4. The extract prepared for Exp. 3 was used. In addition, the effect of precipitation with trichloroacetic acid was investigated. 40 ml. of the extract of Exp. 3, equivalent to 96 g. of pancreas, were treated with an equal volume of 10 % trichloroacetic acid, and the precipitate filtered off. The filtrate was extracted six times with ether to remove trichloroacetic acid, evaporated and then made up to 96 ml. The precipitate represented 6.4 % of the solids taken, and the filtrate 77.4 %.

Exp. 5. 250 g. of minced tissue extracted as in Exp. 1.

Exp. 6. 2 kg. of minced tissue extracted as in Exp. 1; evaporation of the extracts at a bath temperature of 30–40° was continued to dryness and the solid extracted with 500 ml. ether. The residual solid after removal of ether was dissolved in 500 ml. water.

The choline content of each extract was determined by biological assay on the isolated rabbit intestine after hydrolysis and acetylation. It was thus

possible to decide on the amount of choline to be given to the groups receiving choline, in order to assess the non-choline activity of the pancreatic extracts. In addition, the total solids present in each extract, save in that of Exp. 5, were obtained by drying to constant weight at 105°, and the ash content determined. In Exps. 1, 2 and 6 estimations of total N and in the former two of non-protein-N on the filtrate obtained after precipitation with sodium tungstate and H₂SO₄ were made. The results of these analyses are recorded in Table I.

Table I. *The solids of the pancreatic extracts*

Exp.	Yield % fresh pancreas	% of total solids			
		Choline	Total N	N.P.N.	Ash
1	5.5	1.0	10.9	8.0	13.5
2	12.7	2.45	11.8	10.8	8.7
3	10.45	1.64	—	—	10.1
4	10.45	1.64	—	—	10.1
Trichloroacetic acid filtrate	8.08	2.12	—	—	10.8
5	(1.8 mg. choline/g. pancreas)		—	—	—
6	4.64	3.3	9.5	—	16.0

Animal feeding

In Exps. 1, 2 and 6 diets which result in "cholesterol" fatty livers, and in Exps. 3, 4 and 5 diets producing "fat" fatty livers were employed. The diets and the periods of feeding were as follows.

Exp. 1. Caseinogen (alcohol-extracted) 5, cholesterol 2, beef dripping 29, cod liver oil 1, glucose 53, marmite 5 and salts 5 parts; 21 days.

Exp. 2. As in Exp. 1 for 15 days.

Exp. 3. Caseinogen 5, beef dripping 39, cod liver oil 1, glucose 50, salts 5 parts with 10 µg. aneurin per rat per day; 16 days.

Exp. 4. As in Exp. 3 save that the caseinogen was increased to 8 parts at the expense of 3 parts of glucose, while after 7 days it was replaced by commercial egg albumin; 16 days.

Exp. 5. As for the first 7 days of Exp. 4 save that the daily intake of aneurin was increased to 12 µg. and each rat also received 24 µg. lactoflavin; 15 days.

Exp. 6. Commercial egg albumin 8, beef dripping 39, cod liver oil 1, glucose 45, cholesterol 2, salts 5 parts; each rat received 25 µg. aneurin daily; 19 days.

The supplements of pancreatic extract and of choline as chloride were dissolved in the same volumes of water and incorporated in 3-day portions of the dry diets: for the control diets an equal volume of water was used.

At the beginning of the experiments, all the groups except two control groups contained 10 animals (5 ♂, 5 ♀), suitably chosen so that animals of the same weight and sex appeared in each group. At the conclusion of the feeding period, animals which showed a weight loss greater than 20% or any other abnormality were rejected. As a result, when the animals were killed 35 of the groups each contained either 9 or 10 animals, groups 14, 21 and 22 contained 8, and groups 28 and 38 only 7; two control groups 18 and 27, which began as groups each of 20 animals, were finally reduced to 18 and 19 respectively.

Analysis of livers

The methods of investigating the livers and the analysis of the lipoids were as follows. In Exps. 1, 2 and 6 in which the "cholesterol" fatty liver was employed, the total ethereal extract of the pooled livers of each group of animals was prepared. In two of these experiments, it was completely analysed for

phosphatide, cholesterol, cholesteryl esters and glyceride. Since only the glyceride fraction is pertinent to the discussion of the results, the amounts of the total lipoids and glyceride only are recorded (Tables II and V). In Exp. 2 a detailed analysis was not made, fatty acids and unsaponifiable matter only being determined after saponification. In this experiment the total fatty acids and the total lipoids only are recorded (Table III). Previous experience has shown that for all practical purposes differences in the total fatty acid contents of the livers of the different groups may be regarded as differences in glyceride. In Exps. 3-5 in which the "fat" fatty liver was investigated the livers of all the animals were individually analysed by the saponification method for their content of fatty acids and unsaponifiable matter. The "fat" contents of the different groups of these three experiments recorded in Table IV are thus the mean values of the fatty acids and unsaponifiable matter of the individual animals of each group.

Food intake

In Exp. 1 the daily food intake of all the animals was measured, and in Table II the amounts of pancreatic extract or of choline recorded are the actual amounts ingested by the different groups. In Exps. 2-6 the daily food consumption was not determined and the amounts of pancreatic extract or choline recorded in Tables III-V have been calculated on the basis that each rat consumed 8 g. of diet per day. Much previous experience suggests that this is a reasonable figure for purposes of calculation for animals of the weight used: further, since the average weight losses in the various groups of each experiment were similar, no serious error will be introduced in using such calculated values for comparative purposes, as will be shown later. In the tables the amount of pancreatic extract is recorded as the daily intake of the weight of pancreas from which it was derived ("pancreas equivalent"), and also as the daily intake of its contained solids ("actual weight"). The "extra choline intake" represents the choline additional to that present in the basal diet received by the different groups either in the pancreatic extract or as administered choline chloride.

RESULTS

When a liver becomes fatty, the percentage of fat in it increases and at the same time its size increases. It is thus possible to discuss the results from both these points of view, but since the calculated weight of "fat" in the liver of the 100 g. rat reflects both these changes, it gives a more complete picture of the results. Accordingly, the results will be discussed only on the basis of the weight of the constituents in the liver of the 100 g. rat, although both the liver size and the percentages of "fat" are recorded in Tables II-V.

In Table II the extra choline intakes of groups 2 and 3 which received pancreatic extract, appear as negative values, because the food consumption of these groups was less than that of the others, the basal diets in both Exps. 1 and 2 being relatively rich in choline (32.5 mg. per 100 g.), since they contained marmite.

The results of groups 2 and 3 show that the administration of the extract derived from 0.44 and 0.08 g. of pancreas has lowered the amount of glyceride in the livers from the control figure of 1.96 g. to 0.88 and 1.11 g. respectively. These marked decreases show clearly the considerable effect of the pancreatic extract in preventing fat deposition in the liver. Further, this effect is not due to choline, for the choline intakes of these two groups were actually less than

Table II. *Exp. 1. Comparative activities of choline and pancreatic extracts on glyceride deposition in the "cholesterol" fatty liver*

Group	Supplement administered	Average initial body wt. g.	Wt. loss % initial body wt.	Food intake g.	Extract administered			Liver % final body wt.	Total lipid		Glyceride	
					Actual wt./rat/day mg.	Pancreas equivalent g.	Extra choline intake/rat/day mg.		% fresh liver	In liver of 100 g. rat g.	% fresh liver	In liver of 100 g. rat g.
1	—	180	6	9.0	—	—	—	6.3	37.3	2.34	31.3	1.96
2	Pancreatic extract	176	7	7.4	24.4	0.44	-0.3	4.7	24.6	1.14	19.0	0.88
3	Pancreatic extract	165	4	8.0	4.4	0.08	-0.3	5.0	29.2	1.46	22.1	1.11
4	Choline	167	2	9.1	—	—	9.2	4.0	13.9	0.54	7.4	0.29
5	Choline	167	2	8.5	—	—	4.1	4.6	20.3	0.93	13.7	0.63

Table III. *Exp. 2. Comparative activities of choline and pancreatic extracts on total fatty acid content of "cholesterol" fatty liver*

Group	Supplement administered	Average initial body wt. g.	Wt. loss % initial body wt.	Actual wt./rat/day mg.	Extract administered			Liver % final body wt.	Total lipid		Fatty acids	
					Actual wt./rat/day mg.	Pancreas equivalent g.	Extra choline intake/rat/day mg.		% fresh liver	In liver of 100 g. rat g.	% fresh liver	In liver of 100 g. rat g.
6	—	143	0	—	—	—	—	5.9	30.9	1.83	25.1	1.48
7	Pancreatic extract	139	4	93	0.74	—	2.3	4.3	18.6	0.80	13.0	0.56
8	Pancreatic extract	161	4	46	0.37	—	1.2	4.7	22.8	1.07	17.3	0.81
9	Pancreatic extract	146	5	24	0.19	—	0.6	5.2	30.3	1.58	24.1	1.26
10	Pancreatic extract	154	6	9	0.07	—	0.2	5.5	34.4	1.89	28.8	1.58
11	Pancreatic extract	149	8	1	0.008	—	0.02	5.0	32.5	1.64	26.2	1.32
12	Choline	153	4	—	—	—	5.5	4.3	18.7	0.80	13.7	0.58
13	Choline	161	13	—	—	—	3.9	4.0	18.8	0.75	13.6	0.54
14	Choline	156	9	—	—	—	3.1	4.1	15.2	0.62	10.6	0.53
15	Choline	166	7	—	—	—	2.3	4.5	21.6	0.96	16.7	0.74
16	Choline	162	7	—	—	—	1.6	4.6	24.8	1.15	19.6	0.91
17	Choline	158	5	—	—	—	0.8	5.7	32.2	1.83	26.8	1.52

that of the control group. Since the greater decrease in group 3, 1.08 g., is less than the 1.33 g. decrease caused by 4.1 mg. choline in group 5, it is impossible accurately to assess the activity of the extract in terms of choline. If the arbitrary assumption be made that the preventive action of choline is linear over this range of liver fat percentages, then the extract from 0.44 g. pancreas has been as effective as $\frac{108}{133} \times 4.1$ mg. choline. The pancreatic extract thus has an activity equivalent to 757 mg. choline per 100 g. pancreas, whereas its contained choline represents 55 mg. per 100 g. i.e. its activity is about 14 times that of its contained choline. While the assumption as to the linear nature of the effect is probably not strictly accurate, the results of this experiment appear conclusive in showing the presence in the pancreatic extract of an active substance other than choline. It is to be mentioned also that if the decrease in group 3 were equated with that of group 5 in the same manner as was done for group 2, the activity of the pancreatic extract would have appeared considerably greater even than that found above.

The results in Table III illustrate the limitations of the experimental method, and the caution which is necessary in interpreting the results. The livers of the control group contained 1.48 g. fatty acid; this decreases to 0.56 g. with the highest dose of extract (pancreas equivalent 0.74 g.) and with succeeding doses the amount increases until in group 10 with a pancreas equivalent of 0.07 g., it is 1.58 g., a figure very similar to that of the control group. In the last of the pancreatic groups, however, the amount falls to 1.32 g. yet clearly it should be identical with that of the control group, since the amount of the pancreas equivalent was negligibly small in this group. This illustrates the variations which are to be encountered even when groups of 10 animals are used and emphasizes the unreliability of attempts to equate the effect of any particular dosage of pancreatic extract unless it has a pronounced effect in decreasing the amount of liver fat.

The choline groups, 12-17, illustrate another difficulty, namely that it is not possible to demonstrate clearly changes caused by small successive decreases in the amount of choline administered, unless the doses employed are near the limiting effective amounts of 2-3 mg. Thus each animal of groups 12, 13 and 14 received daily 5.5, 3.9 and 3.1 mg. choline respectively; yet the amounts of fat in the livers of these three groups, 0.58, 0.54 and 0.53 g. are the same. It is thus impossible to differentiate between the effects of 3.1, 3.9 and 5.5 mg. choline. The decreasing effects of amounts of choline lower than these appear, however, in groups 15, 16 and 17, in the last of which with an intake of only 0.8 mg. the amount of fatty acids in the liver, 1.52 g., has returned to the control figure 1.48 g.

The results of pancreatic group 7, therefore, could be equated with those of either of the choline groups 12, 13 or 14. In order to obtain the minimum figure for the activity of the pancreatic extract it is considered wise to equate group 7 with group 14 although it must be emphasized that the effect of the pancreas may be underestimated by so doing. If this be done the extract from 0.74 g. pancreas and containing 2.3 mg. choline has an action equal to 3.1 mg. choline. A second value may be obtained from the results of group 8, where the liver fat is 0.81 g., which lie between those of groups 15 and 16 in which the amounts are 0.74 and 0.91 g. respectively. If an intermediate value be taken, a pancreas equivalent of 0.37 g. and containing 1.2 mg. choline is equivalent in action to 2.0 mg. choline. From the results of groups 7 and 8, therefore, the pancreatic extract in this experiment had an action equal to that of 419 and 541 mg.

Table IV. *Comparative activities of choline and pancreatic extracts on total "fat" content of "fat" fatty liver*

Group	Supplement administered	Average initial body wt. g.	Wt. loss % initial body wt.	Extract administered			Extra choline intake/rat/day mg.	Liver % final body wt.	"Fat" % fresh liver	"Fat" in liver of 100 g. rat g.
				Actual wt./rat/day mg.	Pancreas equivalent g.	Exp. 3				
18	—	154	12	—	—	—	—	4.7	21.7	1.07
19	Pancreatic extract	156	12	100	0.96	—	1.6	3.7	9.2	0.34
20	Pancreatic extract	155	9	80	0.77	—	1.3	3.8	10.9	0.42
21	Pancreatic extract	150	10	61	0.58	—	1.0	3.8	9.7	0.36
22	Pancreatic extract	158	14	40	0.38	—	0.65	4.4	12.2	0.55
23	Choline	157	12	—	—	—	10.1	3.4	5.0	0.17
24	Choline	156	14	—	—	—	7.4	3.8	7.2	0.26
25	Choline	155	14	—	—	—	5.4	3.6	7.7	0.28
26	Choline	152	13	—	—	—	3.4	4.0	10.1	0.41
27	—	147	13	—	—	Exp. 4	—	4.9	17.0	0.91
28	Pancreatic extract	141	12	50	0.48	—	0.82	4.4	13.3	0.60
29	Pancreatic extract	146	7	25	0.24	—	0.41	4.3	13.5	0.63
30	Pancreatic extract*	139	9	26	0.32	—	0.70	5.0	11.0	0.55
31	Choline	144	7	—	—	—	3.5	4.3	9.6	0.41
32	Choline	143	9	—	—	—	2.3	4.5	13.7	0.64
33	Choline	136	10	—	—	—	1.4	4.6	14.6	0.71
34	Choline	146	7	—	—	—	0.7	4.9	15.9	0.78
35	—	158	3	—	—	Exp. 5	—	5.3	16.3	0.98
36	Pancreatic extract	166	2	—	0.24	—	0.4	4.7	14.4	0.71
37	Choline	161	4	—	—	—	3.5	3.9	13.1	0.53
38	Choline	168	3	—	—	—	2.1	4.5	13.5	0.71

* Filtrate from precipitation with trichloroacetic acid.

Table V. *Exp. 6. Comparative activities of choline and pancreatic extracts on glyceride deposition in the "cholesterol" fatty liver*

Group	Supplement administered	Average initial body wt. g.	Wt. loss % initial body wt.	Extract administered			Liver % final body wt.	Total lipids		Glyceride	
				Actual wt./rat/day mg.	Pancreas equivalent g.	Extra choline intake/rat/day mg.		% fresh liver	In liver of 100 g. rat g.	% fresh liver	In liver of 100 g. rat g.
39	—	165	5	—	—	—	8.6	42.3	3.60	36.6	3.11
40	Pancreatic extract	178	7	19	0.40	0.6	6.2	30.66	1.89	24.7	1.53
41	—	167	7	3.7	0.08	0.1	6.5	35.8	2.38	29.8	1.98
42	Choline	164	5	—	—	2.1	5.9	31.6	1.85	25.7	1.51

choline per 100 g. pancreas, while its actual choline content corresponded to 311 mg. Its activity is thus 1.3 or 1.7 times that of its contained choline.

The impossibility of differentiating between the effects of different doses of a supplement unless they differ significantly in amount, which was discussed for groups 12-14 of Exp. 2, is again illustrated in Exp. 3 by groups 19-21 receiving pancreatic extract and groups 23-25 receiving choline. In order not to favour the pancreatic extract choline group 26 is equated with group 20, even though the amount of fat is lower in group 21, which received a smaller pancreas equivalent. Calculating as previously described, the pancreatic extract of Exp. 3 has an action per 100 g. pancreas equal to 442 mg. choline as compared with its content of 171 mg.

The extract used for Exp. 4 was the same as that of Exp. 3. In addition group 30 received the material obtained from the filtrate after precipitating the extract with trichloroacetic acid as described earlier. Both the pancreatic groups 28 and 29 may be equated against the choline group 32. If this be done the extract has an activity equivalent to 479 or 958 mg. choline per 100 g. pancreas. The former of these two amounts is similar to the 442 mg. found for this particular extract in Exp. 3 as described above. These two results, one twice as great as the other, illustrate the limitations of the method when applied to small groups of 10 animals. The trichloroacetic acid filtrate of the pancreatic extract appears to retain all the original activity, for group 30, when equated against group 32, gives an activity equal to that of 712 mg. choline. For the pancreatic extract used in Exps. 3 and 4 the non-choline activity found is thus 442, 479 and 958 mg. choline, and for the trichloroacetic acid filtrate 712 mg.

In Exp. 5, group 36, receiving pancreatic extract, has given a result identical with that of the choline group 38, corresponding to 875 mg. choline as compared with its choline content of 180 mg. During this experiment two other groups of animals received the material of the trichloroacetic acid filtrate obtained by direct extraction of the original pancreas after removal of the trichloroacetic acid as described for Exp. 4. The results which are not recorded in the tables suggested that this method of direct extraction of the pancreas was not as effective as the alcoholic extraction used in the other experiments.

In Exp. 6, pancreatic group 40 may be equated with choline group 42. The activity of this extract is thus 525 mg. choline per 100 g. pancreas; its choline content was 153 mg.

DISCUSSION

Data are presented in Table VI which emphasize the more important points which need discussion. The weight of pancreas equivalent to the volume of extract administered and the actual solids of the ingested extract are recorded as before. Column (c) records in terms of choline the calculated non-choline activity of the pancreatic extract administered, and is obtained by subtraction of the "extra choline intake" of the pancreas groups of Tables II-V from the particular choline dosage with which each extract dosage was equated. Column (d) then expresses the non-choline activity of each extract referred to 100 g. pancreas, expressed in terms of mg. choline.

During the six experiments separate extracts were used in Exps. 1, 2, 5 and 6; a fifth extract was used both for Exps. 3 and 4, and also a trichloroacetic acid filtrate of this extract in Exp. 4. As shown in Table VI, it is possible to assess the activity of the five extracts in terms of choline on eight occasions. After allowing for the actual choline contents of the extracts, which had been previously determined in every case, all the extracts had an action in preventing

Table VI. *Assessment of the activity of pancreatic extracts in terms of choline*

Exp.	Extract administered		Choline equivalent of the non-choline activity of the pancreatic extract administered mg. (choline) (c)	Non-choline activity of extract/100 g. pancreas mg. (choline) (d)
	Pancreas equivalent/rat/day g. (a)	Solids mg. (b)		
1	0.44	24	3.3	757
2	0.74	93	0.8	108
	0.37	46	0.8	216
3	0.77	80	2.1	273
4	0.48	50	1.5	308
	0.32*	26	2.1	670
5	0.24	—	1.7	704
6	0.40	19	1.5	375

* Filtrate from precipitation with trichloroacetic acid.

fat deposition in the liver varying from an equivalent of 108 mg. in Exp. 2 to 757 mg. in Exp. 1, when expressed as mg. choline per 100 g. pancreas (column (d), Table VI).

The method of assay is not as accurate as could be desired but care has been taken in each case to assess the activity of the pancreatic extracts at a minimum. For example, in Exp. 2, group 7 was equated with group 14, giving a non-choline activity of 108 (Table VI). Groups 12 and 13 however both gave the same amount of fat in the liver as group 14, and had group 7 been equated with either of these, the non-choline activity would have been assessed as 432 and 216 mg. choline per 100 g. pancreas, amounts four times and twice as great. Similarly, if in Exp. 3 group 21 and not group 20 had been equated with choline group 26, the activity found would have been 586 mg. instead of 442 mg. choline per 100 g. pancreas. The average value for the non-choline activity in the eight assays of the 5 extracts corresponds to 426 mg. choline per 100 g. pancreas. It might be objected that since the food intakes were not measured save in Exp. 1, only the results of that experiment are valid, for if the choline groups consumed less food than the 8 g. daily on which the assays are based, clearly the activities of the pancreatic extracts would be assessed at too high a figure. This objection cannot be sustained. In the first place, the food intakes of the choline groups in Exp. 1 are higher than those of the pancreatic groups, and it has been our general experience that these fatty diets are better consumed when choline supplements are added. Secondly the food intakes of the choline groups necessary to reduce the non-choline activity of the extracts to zero, may be calculated. Such calculations show that the amounts would have to be so low as 5.9 and 4.8 g. in Exp. 2, 3.1 g. in Exp. 3, 2.8 and 1.4 g. in Exp. 4, 1.5 g. in Exp. 5 and 2.3 g. in Exp. 6. Since the weight losses of the choline groups were similar to those of the pancreas groups, such values for food intake are clearly impossible. We think it probable that the assumption of a uniform food intake for all the groups has in fact operated against the pancreas rather than in its favour, as it would have done had it been made in Exp. 1.

It is of interest to calculate the percentage of the total activity of each extract which is due to its choline content. This value ranges from 0% in Exp. 1 to 74% in Exp. 2 with a mean value of 35%. Thus the choline content of the

extracts accounts for only one-third of their activity. Since this is, as already pointed out, a minimum estimate, the presence of a non-choline factor in pancreas appears established, thus extending the claims of Dragstedt *et al.* [1936, 1, 2] to the dietary fatty liver.

While the *non-choline* activity of the extracts, 426 mg., is about $1\frac{1}{2}$ times the actual choline content of the whole pancreas, it is to be admitted that this represents a low degree of activity if the substance is a hormone. The probable explanation is that the method of extraction is inefficient, but in view of the conflicting evidence now to be reviewed, we thought it better to establish the presence of the substance before attempting to improve its extraction.

Since proteins exert a preventive action in liver fat deposition [Channon & Wilkinson, 1935; Beeston *et al.* 1935; Best *et al.* 1936; Channon *et al.* 1938], it is possible that the extra activity of the extracts may be due to their content of protein. In Table I it is shown that the solids of the extracts of Exps. 1 and 2 contained 10.9 and 11.8% total N, and 8.0 and 10.8% non-protein-N respectively. In Exp. 1 the protein-N was, therefore, 2.9% corresponding to 18.1% of protein, and of the 24 mg. actual solids administered 4.3 mg. were protein. If the activity were due to protein it may be calculated that the protein would possess an activity equal to 767 mg. choline per g. Similar calculation shows that in Exp. 2, 1 g. of protein would have the activity of 138 mg. choline. Of many proteins studied [Channon *et al.* 1938] none has an activity significantly greater than that found by Beeston *et al.* [1936] for caseinogen, i.e. 7 mg. choline per g. Clearly, therefore, a protein cannot be responsible for the non-choline effect of the pancreas unless it possesses an activity vastly greater than that of any yet encountered. Further in a single observation in Exp. 4 the trichloroacetic acid filtrate was found to retain all the original activity of the extract. It must not be overlooked, however, that the active substance may have been present in the pancreas originally as part of a protein molecule and liberated by enzymic action during the earlier stages of preparation of the extracts.

The work of Dragstedt and his colleagues has been much criticized, and failure to repeat his results both on depancreatized dogs and on rats has been reported by a number of workers. Chaikoff & Kaplan [1937] have drawn attention to the impossibility of making comparative studies of the effects of choline and pancreatic extracts in any quantitative manner by the histological methods which Dragstedt employed. They have further shown that the fat distribution in the livers of depancreatized dogs varies from lobe to lobe, and therefore that results based on histological examination of biopsy samples are unreliable. Using depancreatized dogs, Kaplan & Chaikoff [1937] were not satisfied that pancreatic extracts had an effect other than that of their choline content. They point out that until properly designed experiments have been carried out in which the choline contents of the extracts are determined and taken into account in any assays, the results of Dragstedt *et al.* [1936, 2] cannot be accepted.

Aylward & Holt [1937] fed groups of rats on a diet causing fatty livers; other groups received the same diet with the addition of raw pancreas or corresponding amounts of choline calculated from the figures for ox pancreas recorded by Fletcher *et al.* [1935]. They concluded that the effect of raw pancreas was adequately explained by its choline content. Study of their results, however shows that the amounts of pancreas and choline fed were such that the "fat" percentage in the livers was in our opinion only sufficiently high at one pancreas dosage for any adequate comparison to be made between the effects of the two

supplements; in addition they based their conclusions on the percentage of fat in the livers only and took no account of the changes in liver size. In this one result, calculation from their data of the amount of fat in the liver of the 100 g. rat shows that 1 g. of pancreas had an action approximately equal to that of 3.5 mg. choline which may be contrasted with the 2.3 mg. choline per g. of ox pancreas reported by Fletcher *et al.* [1935]. Owing to the possible lipotropic effect of the proteins of the pancreas fed, this single result provides but slight evidence as to the existence of an active pancreatic factor other than choline, especially since raw pancreas was fed, and any active non-choline compound in it may have been inactivated by enzyme action. MacKay [1937] administered extracts prepared by Dragstedt's method to rats receiving a diet causing fatty livers in amounts equivalent to 1.2–30 g. of pancreas per rat per day. The livers of the control animals contained 21.5 % fat, and no significant change occurred until the animals received the equivalent of 3 g. of pancreas per day, when it fell to 13.2 %. The author deduced that the pancreatic extract was active in preventing fat deposition, and, holding the view that the extract could not contain enough lecithin or choline to give the experimental results quoted, attributed its action to a non-choline factor. As shown in Table I, however, such extracts may contain much choline, and indeed in a later paper [MacKay & Barnes, 1938], the same worker attributes half the activity of such extracts to their contained choline. Since the choline contents of the extracts were not determined, while in addition the extracts from very considerable amounts of pancreas were found necessary in order to demonstrate an effect, it is difficult in our opinion to draw any conclusions from these results. MacKay & Barnes [1938] reported further experiments in which extracts equivalent to very much smaller amounts of pancreas (up to 1 g. per rat per day) were found active. Finding an approximately equal distribution of activity in the filtrate and precipitate obtained by the addition of 20 vol. of alcohol to their extract, they regarded this as evidence that both the choline and the protein present in pancreatic extracts act to prevent fat deposition in the liver. In contrast to the previous finding [MacKay, 1937] they concluded that there is no specific pancreatic substance, the action of such extracts being the sum of the activities of the choline and protein. Since the crude alcohol precipitate was regarded as protein, and the choline content of neither fraction was estimated, this deduction appears unwarranted. Further, the liver fat percentages in the control groups of two of their three experiments, 8.2 and 7.0, are in our experience far too low to enable any adequate demonstration of the activity of a supplement to be made.

The only existing claim of confirmation of Dragstedt's results occurs in a preliminary note by Shapiro & Wertheimer [1937]. In this work each rat received extract, of undetermined choline content, equivalent to 14–22 g. pancreas per day, in contrast with the maximum of 0.9 g. used in the present work. From diffusion and adsorption experiments, the authors present some evidence which may be taken as indicating the non-identity of choline itself with the active substance.

For the reasons outlined above, it appears to us that the work which has so far been reviewed neither proves nor disproves the existence of a pancreatic non-choline factor influencing liver fat.

Quite recently Dragstedt *et al.* [1938] have adduced further evidence in confirmation of their original findings.

Best & Ridout [1938] have just reported experiments in which they used a pancreatic extract proved active on depancreatized dogs by Dragstedt; their

results lead them to the conclusion that "lipocaic" has no action on rats beyond that to be expected from its content of choline and protein, and that its activity is no more than that of an equal weight of dietary caseinogen, namely 5 mg. choline per g. From the data presented in this paper, values for the lipotropic value of the solids of our extracts may be calculated: such values range from 33 to 138 mg. choline per g. with an average of 72 mg. per g., which is 14 times that accepted by Best & Ridout [1938] for caseinogen. Since our extracts contained appreciable amounts of choline while the "lipocaic" used by Best & Ridout [1938] contained only traces, it would be fairer to calculate the non-choline lipotropic values; these vary from 9 to 138 mg. per g. and average 54 mg. per g., a figure 10 times that found by the Toronto workers. Their opinion is thus in marked and perplexing contrast to that presented in this paper, and in view of the very considerable body of consistent evidence which we offer, we feel that the explanation of the divergence must await further experiment. The only suggestion we offer is that possibly the single preparation of "lipocaic" administered by Best & Ridout [1938] to each of the two groups of rats used had become inactive subsequent to its being tested by Dragstedt; for this reason it would have been of value had it been possible for the preparation after being found inactive on rats at Toronto, to be re-tested for its activity on depancreatized dogs by Dragstedt.

If the conclusions of Best & Ridout [1938] and of Dragstedt *et al.* [1936, 2] are confirmed they will provide a further example of differences between the factors influencing the dietary fatty liver and those affecting the diabetic fatty liver. One such difference is indicated by the original results of Allan *et al.* [1924] and by numerous later studies; the high protein diets on which depancreatized dogs are maintained fail to prevent the development of fatty livers in these animals, yet it is the protein content of diets low in choline which controls the degree of fat infiltration in the rat [Channon & Wilkinson, 1935; Beeston *et al.* 1935; Best *et al.* 1936; Channon *et al.* 1938]. It would, however, be a curious fact if in diabetic dogs developing fatty livers on diets of high protein content, the addition of raw pancreas controlled such fat infiltration by virtue of the additional protein provided, unless the pancreatic protein is of specific nature: alternatively the pancreas must contain some active non-protein substance other than choline.

SUMMARY

1. Dilute alcoholic extracts of pancreas have been prepared according to the method of Dragstedt *et al.* [1936, 2] and the amount of choline in them determined. The action of each extract in preventing fat deposition in the livers of rats has been assayed in terms of choline.

2. Six experiments were carried out on 42 groups of rats, each group consisting of 10 animals. Three of these experiments were concerned with the "cholesterol" and three with the "fat" fatty liver.

3. Eight assays of the activity of the five pancreatic extracts used were made; all of them showed the extracts to possess an ability to prevent fat deposition in the liver greater than could be attributed to their content of choline. The average value for this non-choline activity was equivalent to that of 426 mg. choline per 100 g. pancreas while the choline present in the extracts accounted for only one-third of their activity.

4. The non-choline activity is not accounted for by the protein content of the extracts and it is concluded that there exists in pancreas a substance other than choline which is involved in fat deposition in the liver.

5. Some of the difficulties of assaying pancreatic extracts are pointed out, and the conflicting evidence on the problem of the activity of such extracts is discussed.

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