Lissapol N (Imperial Chemical Industries Ltd., Blackley, Manchester), Stergene (Domestos Ltd., Newcastle-upon-Tyne) and Triton X-100 or Triton A-20 (Rohm & Haas Co., Philadelphia, Pa.) belong to this group of 'alkylaryl polyethoxyethanols'.

The following fluid, which has a specific gravity of 1.033 at 20°, is recommended.

Sodium bromide (anhydrous)	44 g.
Stergene (or Lissapol N)	1 g.
Evans blue	0.3 g.
Water	to 1000 ml.

If filtration is required, sintered-glass funnels should be used as filter paper removes some of the dye by adsorption.

Sodium bromide is used in preference to sodium chloride or potassium chloride because the detergent solution gradually develops a turbidity (not visible in the presence of the dye) on addition of the chlorides, and precipitates are formed on storage in mixtures of detergent, alkali chloride (especially potassium chloride) and Evans blue. Sodium bromide is free from this disadvantage. Commercial sodium bromide usually contains moisture and should be dried at 105–110° before weighing. The figure given for Stergene and Lissapol N refers to the

commercially available syrupy liquid which contains about 70 % water. As Triton X-100 and Triton A-20 are practically water-free, 0.3 g. of either of these detergents is equivalent to 1 g. of the other two. Evans blue, widely used for blood volume determinations (Dawson, Evans & Whipple, 1920), and first recommended for manometer fluids by Bain (1945), has proved more stable than other dyes tested. It is not taken up by the rubber or plastic reservoirs.

The new fluid has a much greater cleansing power than Brodie's solution. It keeps better than the older fluid which sometimes becomes viscous on storage owing to changes in the bile salts. Froth and bubbles formed in the manometer capillary disappear more readily than from Brodie's solution. The greater cleansing power was assessed by placing in the manometer fluid a grease-coated microscopic slide prepared by dipping it into ether containing 1 % lanolin.

SUMMARY

An improved manometer fluid is described in which the bile salts recommended by Brodie are replaced by a non-ionic detergent of the 'alkylaryl polyethoxyethanol' type.

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The Lipid Mobilized by Anterior Pituitary Extract

By J. CAMPBELL AND C. C. LUCAS

Banting and Best Department of Medical Research, University of Toronto

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When certain anterior pituitary extracts are administered to fasting rats a rapid increase in the lipid of the liver and kidneys, and a decrease in that of the other tissues, occurs (Best & Campbell, 1936, 1938). Since more lipid disappears from the depots than accumulates in liver and kidney, it is apparent that increased utilization of lipid accompanies the mobilization. In this study the sources and nature of the lipid mobilized were determined in mice, and certain observations on the distribution of lipid in the body were made. A similar, but less complete, study was made of the effects of the extract when given to fasting rats.

METHODS

Fifty normal adult male mice (raised on a diet of 'Purina' fox chow, supplemented with brown bread and milk) were separated into five groups. The mice were fasted from the beginning of the experiment, but water was always available. Four hours after the beginning of fasting the animals of group 1 (controls) were sacrificed. At this time each animal in groups 2 and 4 (injected controls) was given subcutaneously 9 mg. of an inert protein, bovine plasma albumin (BPA); and each animal of groups 3 and 5 was given 9 mg. anterior pituitary preparation (APP). The mice of groups 2 (BPA) and 3 (APP) were killed 7 hr. after these injections, i.e. 11 hr. after the beginning of the experiment. Mice of groups 4

and 5 were given second injections of BPA and APP, respectively, at this time, and were sacrificed 13 hr. later, i.e. 24 hr. from the beginning of the experiment. The lighter animals were chosen for group 1, since they were to be subjected to the shorter fast. Those in the other groups weighed 20–30 g. The APP was prepared from ox pituitary glands as described by Best & Campbell (1936) and the BPA was obtained from the Armour Laboratories, Chicago. These preparations were dissolved in saline at pH 7.5, and 0.5 ml. solution were injected.

Table 1. *Effect of anterior pituitary preparation on the body lipid of mice*

(Mice, ten per group, were fasted for 4 hr. before the first injection, and thereafter. The lipid composition of the mice is given per 100 g. fat-free solids of the entire bodies.)

Group no.	Injection	Fast after first injection (hr.)	Actual weights of		(g./100 g. fat-free solids of entire body)				
			Entire body (g.)	Fat-free solids (g.)	Lipid	Phospho-lipin	Free cholesterol	Cholesteryl ester	Glyceride
1	None	0	21.4	5.04	37.1	7.10	0.99	0.54	28.5
2	BPA	7	22.5	5.37	38.2	6.45	1.02	0.48	30.1
3	APP	7	25.0	6.07	31.6	5.80	1.00	0.50	24.4
4	BPA	20	22.4	5.59	31.2	6.09	0.98	0.68	23.1
5	APP	20	22.6	5.55	27.9	5.91	1.01	0.48	20.5

The lipid was extracted and analysed according to Best, Lucas, Patterson & Ridout (1946).^{*} The animals were weighed, killed by crushing the occiput and the tissues were combined for analysis as follows: (1) The spleen and gastrointestinal tract. (2) The genital organs, including the testes, epididymis, seminal vesicles and prostate gland. (3) The skin, except for the small portions adherent to the paws and tail. (4) All the adipose tissues visible to the naked eye, as detailed by Reed, Yamaguchi, Anderson & Mendel (1930), including the mesenteric, perirenal, perigenital and other adipose tissues in and about the abdominal cavity, the subcutaneous and interscapular fat tissue and the intermuscular deposits in the pelvic and scapular regions. Reed, Anderson & Mendel (1932) found that, in rats, lipid changes in these tissues tended to be similar. (5) The brain (not including the optic and olfactory nerves or the spinal cord). (6) The trachea, lungs and thymus. (7) The carcass, which consisted of the tissues remaining after those mentioned above and the liver, kidneys and adrenals had been removed. The carcass thus consisted chiefly of muscle and bone plus the heart and peripheral nerves. All these tissues, which are referred to as the 'entire body' constituted about 94% of the live weight; while lost bile, blood and urine accounted for the remaining 6%.

The methods used in the experiment on rats were essentially similar, the differences being described in the text.

^{*} On cooling the clear light petroleum solutions of the initially soluble lipid from intestines and spleen and from brain, an amorphous, white material settled out and tended to redissolve on warming slightly. This small precipitate, when present, was removed by centrifuging. It was subsequently found to be rich in phosphorus and exhibited certain characteristics of sphingomyelin or hydrolecithin (cf. Hunter, 1942). The values presented for the phospholipin of these tissues are consequently, in some cases, low; but the amount lost was a small proportion of the total and does not invalidate the general conclusions drawn.

RESULTS

Effect of APP on the total lipid of all tissues

The total lipid of the entire body was decreased by the administration of APP (Table 1). This indicates that, in mice, increased utilization of lipid occurred under the stimulus of the APP, as had been found previously in rats (Best & Campbell, 1938). The

reduction was most pronounced in the glyceride fraction. The cholesterol and cholesteryl esters of the entire bodies were apparently not influenced significantly by the administration of the APP. Phospholipin appeared to be decreased somewhat, although the differences are of questionable significance. Expressed as percentage of the fresh weights of the entire bodies, the total lipid in groups 1–5, respectively, were 8.74, 9.10, 7.68, 7.78 and 6.85; the fat-free solids similarly expressed were 23.5, 23.9, 24.3, 25.0 and 24.5; and the water contents were 67.8, 67.0, 68.0, 67.2 and 68.8.

Mobilization of lipid

The administration of the APP produced an increase in the lipid of the liver, but only a slight indication of increase in kidney lipid. The lipids of adipose tissue, carcass and skin, in this order, were most heavily drawn upon in the mobilization produced by the APP (Fig. 1). These lipids were decreased by fasting alone, but the administration of the APP accelerated the loss. The genital organs, intestine and spleen, and the lungs and thymus contributed relatively little to the amount of lipid mobilized. The lipid of the brain was apparently not affected.

It appears that these changes in total lipid were due chiefly to changes in glycerides, which were withdrawn from adipose tissue, carcass and skin, and which were deposited in the liver (cf. Figs. 1 and 2). Of the glyceride lost by the tissues mentioned, about one-fifth could be accounted for by that which accumulated in the liver. Presumably about four-fifths had been oxidized.

As a result of the influx of glyceride into the liver following the injection of APP, the phospholipin (expressed as percentage of the fresh weight) was decreased. Further studies are required before conclusions can be drawn regarding changes in the absolute amounts of phospholipin or of cholesterol in the tissues under these conditions.

the skin contained about one-half, and the liver the next largest amount (5-6%). The remaining 11% was distributed among the other tissues.

Somewhat more than one-half of the phospholipin of the body occurred in the carcass; the liver contained the next largest portion (15%), the brain contained about 6-7% and the skin a similar

Fig. 1.

Fig. 2.

Figs. 1 and 2. The total lipids and glycerides of mouse tissues, g./100 g. fat-free residues of the entire animal. The fasting mice were injected with APP, $\odot$ ---- $\odot$; or BPA, $\times$ — $\times$. The tissues indicated are *l*, liver; *k+a*, kidney and adrenal; *a*, adipose tissues; *c*, carcass; *s*, skin; *g*, genital organs; *i+s*, intestine and spleen; *l+t*, lungs and thymus.

Distribution of lipid

The distribution of lipid in the bodies of mice of group 1 is shown in Table 2, the values for the other groups being similar. The carcass and the adipose tissues each contained about one-third of the total lipid of the entire body. Of the remaining one-third

amount. The largest proportion of the free cholesterol (41%) occurred in the carcass; the skin, although second in this respect, contained less than one-half as much. The skin contained about 60% of the cholesteryl esters and the carcass about 25%. The adipose tissues contained about 40% of the glycerides of the entire body, the carcass about 28%

Table 2. *The distribution of lipid in mice*

(Group 1, fasted 4 hr. The values are given as percentages of the total amounts of each of the components in the entire animal, i.e. each vertical column totals 100%.)

Tissue	Wet weight	Fat-free solids	Water	Total lipid	Phospholipin	Free cholesterol	Cholesteryl ester	Glyceride
Carcass	54.3	60.9	54.7	33.9	57.1	41.1	26.5	28.0
Adipose tissues	6.59	3.10	4.48	31.8	5.40	5.16	0.55	40.0
Skin	13.5	17.2	11.7	17.7	6.30	16.6	59.8	19.8
Liver	6.50	6.15	6.70	5.20	15.1	6.80	3.11	2.60
Intestine and spleen	12.25	8.20	14.7	3.53	2.40	11.1	4.80	3.50
Genital organs	1.97	1.18	2.09	3.25	1.87	1.74	1.47	3.61
Kidneys and adrenals	1.84	1.28	2.02	1.77	3.97	4.9	1.10	1.20
Brain	1.68	0.87	1.93	1.92	6.11	10.7	1.94	0.70
Lungs and thymus	1.42	1.03	1.62	0.87	1.76	2.0	0.74	0.60

Table 3. *Composition of the tissue lipids*

(Group 1, mice fasted 4 hr.)

Tissue	Percentage of total lipids of tissue			
	Phospholipin	Free cholesterol	Cholesteryl ester	Glyceride
Carcass	32.4	3.27	1.13	63.6
Adipose tissue	3.28	0.434	0.025	96.6
Skin	6.90	2.55	4.90	85.8
Liver	57.1	3.57	0.88	38.4
Intestine and spleen	13.4	8.60	2.00	76.5
Genital organs	11.1	1.43	0.72	86.7
Kidneys and adrenals	42.4	4.27	0.76	52.5
Brain*	54.3	14.9	1.42	29.3
Lungs and thymus	38.8	6.17	1.35	53.7

* The averages of groups 2-5.

Table 4. *Rats, female, fasted and injected daily for 3 days*

Substance injected	Body weight, initial (g.)	Fatty acids (mg./100 g. body wt.)			
		Carcass	Intestine	Liver	Kidney
APP	176	4460	330	504	41
Saline	185	5250	441	131	35
Change		- 790	- 111	+ 373	+ 6
		- 901		+ 379	
Unaccounted for		- 522			

and the skin about 20 %. Greater amounts of lipid, free cholesterol, phospholipin and water were found in the carcass than in any of the other tissues.

Composition of the lipid

Of the lipid of the carcass, somewhat less than two-thirds was present as glyceride and one-third as phospholipin; while the free and bound cholesterol together amounted to about 4 % (Table 3). The lipid of skin was rich in glycerides (86 %), but its outstanding characteristic was a high concentration of cholesteryl ester, which exceeded the concentration of free cholesterol. The lipids extracted from liver and from kidney and adrenals were conspicuously rich in phospholipin.

It would appear that the rate of mobilization of lipid may be related to the glyceride content of the

tissue. For example, the percentage of glyceride in the lipids of adipose tissue, genital organs, skin and carcass were 97, 87, 86 and 64 respectively, while the losses of lipid from each of these tissues under the influence of the APP during 11 hr. fast, when expressed as the percentage of lipid in the fasted controls, were 32, 26, 24 and 12, respectively. The corresponding losses of glycerides, expressed as percentages of the glycerides in the controls, were 32, 28, 30 and 9, respectively.

Fatty acid mobilization and utilization in rats

The following experiment demonstrates (in confirmation of Best & Campbell, 1936), that the injection of APP produces increased mobilization and utilization of lipid in fasting rats. Adult rats, 10 per group, were fasted and injected daily for 3 days with

APP, while the control rats were given equivalent amounts of saline. The total crude fatty acids (fatty acids plus unsaponifiable lipid) were then determined (Table 4). In the group given APP, 379 mg. crude fatty acids per 100 g. initial body weight were gained by liver and kidney, while 901 mg. were lost by the other tissues. The difference of 522 mg. had presumably been utilized.

DISCUSSION

It was demonstrated by Barrett, Best & Ridout (1938) that the administration of APP to fasting mice caused rapid mobilization of lipid from the depots with an accumulation of lipid in the liver. Stetten & Salcedo (1944) demonstrated this effect in rats. The results of the present study in mice and rats support and extend these findings.

The glyceride fraction was principally affected in the mobilization of lipid produced by the anterior pituitary stimulation. Glycerides were removed from the depots, were deposited in liver, and were utilized to a greater extent than any other lipid fraction examined. While no attempt is made to explain the mechanism of lipid mobilization, it

seems probable from the evidence available that the rate of mobilization from a tissue may be related to the glyceride content of that tissue.

The liver and, to a much less extent, the kidney were the only structures of those examined whose lipid contents increased during the period of lipid mobilization.

SUMMARY

The mobilization, accumulation in the liver and utilization of lipid in fasting mice injected with an anterior pituitary preparation involves chiefly the glycerides. The amount of lipid transferred from a tissue under these conditions appears to bear a relation to the glyceride content of the lipid of the tissue. The rat appears to respond similarly to treatment with anterior pituitary preparation.

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The Effect of a High-Sucrose Diet on the Calcium and Phosphorus Content of the Enamel and Dentine of Rat Incisors

By R. L. HARTLES

Biochemistry Department, School of Dental Surgery, University of Liverpool

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So far as is known no investigation has been carried out to determine the effect of a large excess of a major dietary constituent on the process of mineralization. The work reported here was concerned with investigating the effect of a diet containing 67% sucrose on the calcium and phosphorus content and the calcium/phosphorus ratio of rat incisors. In recent years such a diet has been used for the experimental production of dental caries in the white rat, the hamster and the cotton rat (Sognnaes, 1948*a, b*; Shaw, Schweigert, Elvehjem & Phillips, 1944). It is of interest, therefore, to know if teeth produced on

a high-sugar diet differ chemically from those of animals receiving a typical stock laboratory ration, when both diets contain adequate amounts of the known vitamins and necessary minerals. Work in the past appears to have been concerned, in the main, with deficiencies of vitamins and minerals, several factors having been shown to influence the structure and composition of growing teeth. In general, however, dietary deficiencies need to be much more severe to influence the composition of the teeth than similarly to affect bone. For example, diets which cause severe rickets may produce only slight changes