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ADIPOSE TISSUE AND HEAT PRODUCTION IN THE NEW-BORN OX (*BOS TAURUS*)

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SUMMARY

1. A histological examination of adipose tissue from 1- and 6-day-old calves showed a structure typical of white adipose tissue and no evidence of brown adipose tissue.

2. Infusion of noradrenaline (1·0 μg/kg.min I.V.) into 1-to 6-day-old calves affected heart rate and respiratory rate but did not increase heat production, rectal temperature, skin temperature or skin evaporative loss.

3. Cold exposure led to shivering and an increased oxygen consumption in the 6-day-old calf.

4. Blood samples taken from 6-day-old calves in the cold ($-1°$ C) appeared to have a higher proportion of unesterified fatty acids in the total blood lipid than samples taken in an environment of 20° C, but no change in fatty acid composition was found.

5. It has been concluded that there is no non-shivering thermogenesis in the young calf.

INTRODUCTION

The new-born rabbit, kitten, pig, rat, human, lamb and ox increase their oxygen consumption and, therefore, their heat production on exposure to cold (Adamsons, 1959; Hill, 1959; Mount, 1959; Taylor, 1960; Brück, 1961; Alexander, 1961; Gonzalez-Jiminez & Blaxter, 1962). The new-born lamb shivers, but in some other species, e.g. the rat, shivering is not observed until the animals are older. The early post-natal type of heat production is termed non-shivering thermogenesis. New-born rats, kittens and rabbits (Moore & Underwood, 1963) increase their heat production when injected with noradrenaline, and this has suggested that in cold environments non-shivering heat is produced as a result of secretion of this hormone.

The brown adipose tissue of the new-born rabbit is the tissue which

responds to noradrenaline. The temperature and oxygen consumption of the brown fat increases when the animal is injected with noradrenaline or exposed to cold (Dawkins & Hull, 1964; Heim & Hull, 1966). Brown adipose tissue has also been found in new-born infants (Dawkins & Scopes, 1965) and new-born guinea-pigs (Brück & Wunnenberg, 1965) and these animals also increase their heat production after an injection of noradrenaline.

The object of the present work was to determine the type of adipose tissue that is present in the young calf and to find whether the increased heat production of the calf when exposed to cold is due to shivering or to non-shivering thermogenesis.

METHODS

Animals. Fourteen male Ayrshire calves aged from 12 hr to 7 days and weighing from 27·0 to 37·6 kg were used. They were kept in rooms in which the ambient temperature ranged from 9 to 16° C, and were fed approximately 2 l. of cows' milk twice daily. Each animal was used for only one experiment which was carried out in a climatic chamber (Findlay, McLean & Bennet, 1959).

Temperature and evaporation from the skin. Rectal and skin temperatures were measured with a thermocouple system accurate to $\pm 0{\cdot}1°$ C. Evaporative loss from the skin was determined with a ventilated capsule (McLean, 1963).

Heart and respiration rates. These were obtained with a stethoscope.

Cannulation. A polythene cannula, 2 mm in external diameter, was placed aseptically in the external jugular vein under local anaesthesia immediately before the experiment. When used for drug infusion the cannula was first filled with heparinized 0·9 g NaCl/100 ml. solution, but when used for withdrawing blood samples it was filled with a 3·8 g trisodium citrate/100 ml. solution.

Drugs. (−)-Noradrenaline bitartrate (Levophed, Bayer Products Co.) was dissolved in 0·9 % NaCl solution for infusion. Weight of drug is expressed in terms of the base.

Heat production. Heat production was estimated by measuring oxygen consumption, using an open circuit with a ventilated face mask, as described by Findlay & Thompson (1968).

Blood lipids. A blood sample (20 ml.) was taken from each animal after 2 hr in a warm environment and again after 2 hr in a cool environment or vice versa. The total lipids were extracted from each plasma sample by the technique of Folch, Lees & Stanley (1957) as adapted by Nelson & Freeman (1959) and stored in a chloroform–methanol mixture (2:1, v/v). Individual lipid groups were then separated by fractionation on thin layer chromatoplates of Merck silica gel with a solvent system of ether–hexane–formic acid mixture (20:80:1 v/v). The solutions of the total lipid extracts and the solvent mixture used in the development of the chromatogram contained 4-methyl-2,6-di-*t*-butyl phenol (0·1 g/100 ml.) as an antioxidant (Wren & Szczepanowska, 1964). After development the chromatograms were sprayed with a solution of dichlorofluorescein (0·1 g/100 ml.) and 4-methyl-2,6-di-tert-butyl phenol (0·1 g/100 ml.) in methanol–water mixture (95:5 v/v). The positions of the various lipid bands on the plate were located by viewing them under ultraviolet light. As described by Goldrick & Hirsch (1963), the lipid fractions were then eluted from the silica gel with ether (for cholesterol esters, triglycerides and free fatty acids) or with methanol (for phospholipids), and the fatty acids in each fraction were converted to the corresponding methyl esters by the method of Stoffel, Chu & Ahrens (1959). The resulting solutions were

finally analysed by gas–liquid chromatography as described by Moore & Williams (1963, 1964).

Adipose tissue sampling. Samples of adipose tissue were obtained from around the heart, the perirenal area and inguinal region of two calves, 1 and 6 days old under pentobarbitone sodium (Sagatal, May & Baker Ltd.) anaesthesia. The tissue specimens were fixed in 12% formol saline, dehydrated in alcohol, cleared in amyl acetate and embedded in paraffin wax. Sections, 3–8 μ thick, were cut and stained with haematoxylin and eosin, orcein-tartrazine and Masson's stain. The two animals were subsequently killed and a post mortem examination of the distribution of fat depots was made.

RESULTS

Adipose tissue. Examination of the distribution of adipose tissue in the body showed that the largest single deposit was around the kidneys. Small deposits were also found around the heart and in the inguinal region. No deposit was found between the shoulder blades or in any other region. Samples from around the heart, kidney and from the inguinal region had the characteristic structure of white adipose tissue (Pl. 1). Each cell had a single large central fat vacuole, and the cytoplasm and nucleus were situated at the periphery.

Noradrenaline infusion. The effect of intravenous infusion of noradrenaline at a rate of 1·0 μg/kg.min for 45 min in an environment of 20° C is shown in Fig. 1. During the initial control period NaCl solution (0·9 g/100 ml.) was infused and the mean heart rate was 96 beats/min. With noradrenaline infusion there was an initial significant decrease in mean heart rate of $16{\cdot}3 \pm 5{\cdot}4$ (S.E.M.) beats/min ($P < 0{\cdot}05$). On continued infusion, however, there was an increase in heart rate which persisted after substitution of saline for noradrenaline. The average respiration rate during noradrenaline infusion was $11{\cdot}2 \pm 3{\cdot}94$ (S.E.M.)/min higher than during the preceding saline infusion ($P < 0{\cdot}05$). Oxygen consumption was slightly, but not significantly, higher during noradrenaline infusion, the difference from the saline infusion period averaging $1{\cdot}3 \pm 0{\cdot}77$ (S.E.M.) ml./kg.min ($P > 0{\cdot}1$). There was no change in the rectal or ear skin temperatures, or in evaporative moisture loss from the skin with infusion of noradrenaline.

Oxygen consumption and environmental temperature. Two calves, both aged 6–7 days, were exposed to various environmental temperatures between -1 and $+30$° C, and their rates of oxygen consumption measured. Exposure to any one temperature usually lasted for an hour before constant readings were obtained, so that the complete resting of each animal required a period of 2 days. The results for one of these animals are shown in Fig. 2.

This animal was fed before the experiment and its oxygen consumption at 26° C, which appeared to be a thermoneutral temperature, averaged

9·0 ml./kg.min. Oxygen consumption increased with decreasing temperature reaching a peak of 14·7 ml./kg.min at 3° C. Between 26 and 3° C, therefore, oxygen consumption increased by an average of 0·25 ml./kg.min

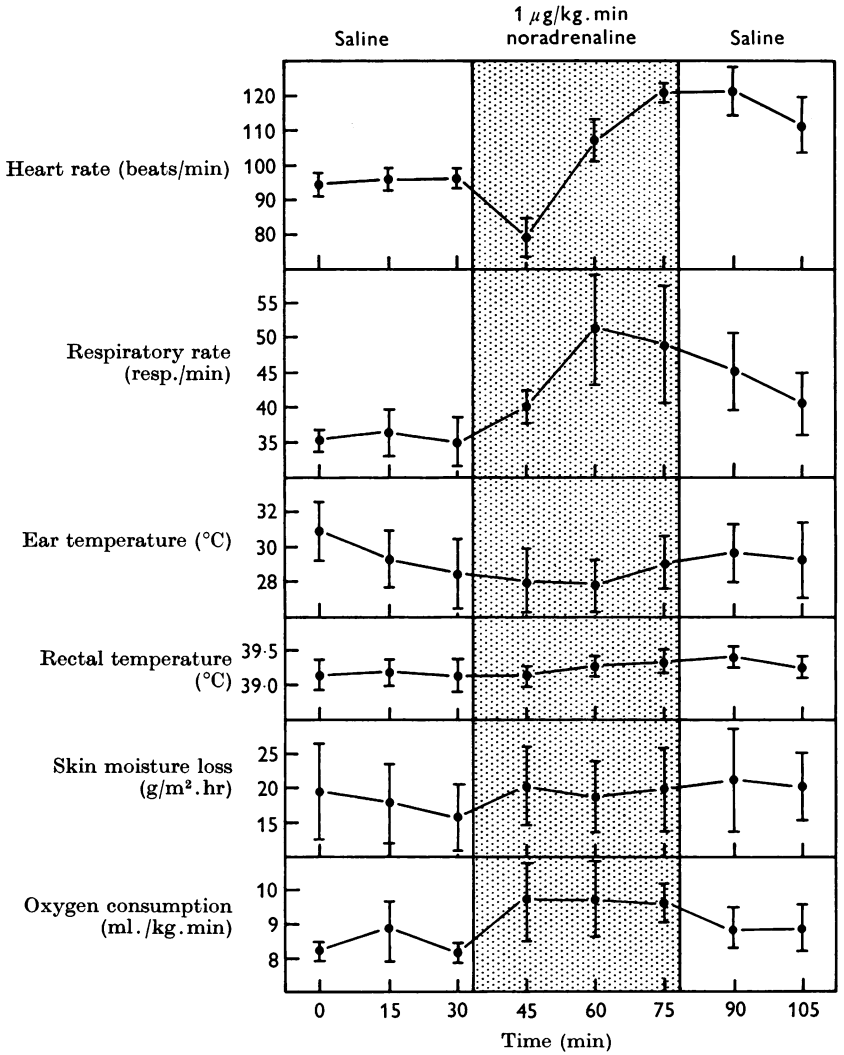

Fig. 1. The effect of noradrenaline (1·0 μg/kg.min I.V.) at 20° C, on six calves aged between 1 day and 6 days (mean ±S.E.).

for each degree drop in ambient temperature. Similar results were obtained from the other animal, although the peak oxygen consumption was slightly lower. Shivering, which was particularly noticeable between −1 and +3° C, occurred in both animals.

Blood lipids in different environments. Having determined at what environmental temperatures the new-born calf increases its oxygen consumption, blood samples were taken from four 6-day-old calves 2 hr after exposure to a warm, and to a cold, environment. The samples contained mainly cholesterol esters and phospholipids with very much smaller concentrations of triglycerides, unesterified fatty acids (U.F.A.) and free cholesterol. Only traces of mono- or diglycerides were present. Subjective viewing of thin layer chromatographic plates indicated that in

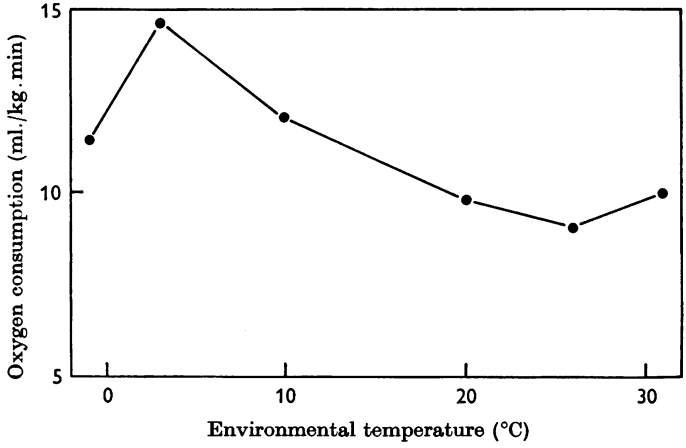

Fig. 2. The oxygen consumption, at different environmental temperatures, of a calf aged 6–7 days.

TABLE 1. Mean fatty acid compositions* of plasma lipid fractions from four 6-day-old calves after 2 hr in environments of 20° C and −1° C

	Cholesterol esters		Triglycerides		Unesterified fatty acids		Phospholipids	
Fatty acid	20° C	−1° C	20° C	−1° C	20° C	−1° C	20° C	−1° C
Palmitic	13·9	14·2	40·9	41·6	30·9	32·5	22·2	23·6
Palmitoleic	8·3	8·2	6·0	6·5	4·7	5·4	1·6	1·2
Stearic	2·4	3·3	12·9	12·7	17·7	15·4	25·4	26·5
Oleic	23·6	23·5	36·4	37·0	40·9	44·8	40·6	37·9
Linoleic	38·4	40·8	1·1	2·1	2·7	1·7	9·7	10·0
Linolenic	9·7	7·5	< 1·0	<1·0	<1·0	< 1·0	<1·0	< 1·0

* Weight percentages of the total fatty acids in each lipid fraction. The fatty acids listed in the table account for about 98% of the total acids present. The remaining 2% was accounted for by trace amounts of fatty acids such as meristic, and various branch chain fatty acids.

samples taken at −1° C the proportion of U.F.A. in the total plasma lipid appeared to be greater than that in samples taken at 20° C. The fatty acid composition of the various lipid groups is illustrated in Table 1 according

to the designation of Farquhar, Insull, Rosen, Stoffel & Ahrens (1959). No change in fatty acid composition was detected when the animal was subjected to a cold temperature.

DISCUSSION

The oxygen consumption per unit body weight at thermoneutrality viz. 8–9 ml./kg.min, found here in fed 6-day-old calves was higher than the basal value (250 ml./m^2.min) reported by Gonzalez-Jiminez & Blaxter (1962) in calves of the same age. Both of these values are lower than the values reported for the new-born of other species, which have a smaller body weight. The oxygen consumption of the new-born pig at thermoneutrality increases from an average of 15·2 ml./kg.min to 21·3 ml./kg.min during the first 2 days of life (Mount, 1959). There is a similar increase in the first 2 days of life in the rat, from 19·7 ml./kg.min to 28·7 ml./kg.min (Taylor, 1960).

The results of Gonzalez-Jiminez & Blaxter (1962), and those obtained in this study, show that the new-born calf exposed to the cold can increase its oxygen consumption by 50–100% which is comparable to the increase that occurs in other species. The results obtained in this study support the view that this increase is brought about by shivering and not by non-shivering thermogenesis.

Brown adipose tissue does not appear to be present in the young calf and white fat was found in only a limited number of sites. Moreover, noradrenaline, which is effective in increasing the heat production of new-born rabbits in concentrations as small as 0·5 μg/kg.min (Scopes & Tizard, 1963), failed to increase the heat production of the calf in concentrations of 1·0 μg/kg.min. This result is in agreement with the findings of J. LeBlanc & L. E. Mount (unpublished data) that the new-born pig, which has very little brown or white adipose tissue, does not exhibit an increased oxygen consumption after subcutaneous injection of noradrenaline. It has been suggested, from studies on the cold-acclimatized adult rat, which has brown adipose tissue, that the skeletal muscles may contribute to the non-shivering thermogenic response to noradrenaline (Jansky & Hart, 1963). The absence of a thermogenic response to noradrenaline in the calf, however, suggests that the brown adipose tissue is the only tissue in the new-born which is stimulated to produce large amounts of heat by noradrenaline.

The most likely way in which noradrenaline could affect heat production is by activation of a tissue lipase (Rizack, 1961) which hydrolyses triglyceride in adipose tissue to glycerol and free fatty acid. In the new-born rabbit the released fatty acids tend to be retained and metabolized in the

brown adipose tissue, whereas in the adult rabbit relatively more fatty acid is released into the circulation from the white adipose tissue (Dawkins & Hull, 1964). When, in the present work, the 6-day-old calf was exposed to cold there was no change in the fatty acid composition of the various blood lipid fractions, but there may have been an increase in the proportion of U.F.A. in the total plasma lipids. The fact that there was no change in fatty acid composition suggests that any effect of cold that there may have been on lipid metabolism was by way of an intensification of the over-all metabolic process already present in the thermoneutral animal rather than by the initiation of a different system of lipid mobilization. It is possible that in the cold, increased amounts of fatty acids are being mobilized and transported in the blood to some other site, perhaps to the skeletal muscles for metabolism during shivering.

New-born animals having brown adipose tissue and exhibiting non-shivering thermogenesis, lose this faculty with age and shivering predominates as the animal becomes older. The lack of non-shivering thermogenesis in the calf and the presence of shivering at birth agrees with other work (Hales, Findlay & Robertshaw, 1968) that in this species an adult-type temperature regulation is present at birth.

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REFERENCES

ADAMSONS, K. (1959). Breathing and the thermal environment in young rabbits. *J. Physiol.* **149**, 144–153.

ALEXANDER, G. (1961). Temperature regulation in the newborn lamb. III. Effect of environmental temperature on metabolic rate, body temperatures and respiratory quotient. *Aust. J. agric. Res.* **12**, 1152–1174.

BRÜCK, K. (1961). Temperature regulation in the new-born infant. *Biologia Neonat.* **3**, 65–119.

BRÜCK, K. & WUNNENBERG, BARBARA (1965). Investigation of the importance of multilocular fat tissue for thermogenesis of the newly-born guinea pig. *Pflügers Arch. ges. Physiol.* **283**, 1–16.

DAWKINS, M. J. R. & HULL, D. (1964). Brown adipose tissue and the response of new-born rabbits to cold. *J. Physiol.* **172**, 216–238.

DAWKINS, M. J. R. & SCOPES, J. W. (1965). Non-shivering thermogenesis and brown adipose tissue in the human new-born infant. *Nature, Lond.* **206**, 201–202.

FARQUHAR, J. W., INSULL, W., ROSEN, P., STOFFEL, W. & AHRENS, E. H. (1959). The analysis of fatty acid mixtures by gas–liquid chromatography: construction and operation of an Ionisation Chamber Instrument. *Nutr. Abstr. Rev.* **17**, supplement.

FINDLAY, J. D., MCLEAN, J. A. & BENNET, R. D. (1959). A climatic laboratory for farm animals. *Heat Vent. Engr* **33**, 95–100.

FINDLAY, J. D. & THOMPSON, G. E. (1968). The effect of intraventricular injections of noradrenaline, 5-hydroxytryptamine, acetylcholine and tranylcypromine on the ox (*Bos taurus*) at different environmental temperatures. *J. Physiol.* **194**, 809–816.

FOLCH, J., LEES, M. & STANLEY, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497–509.

GOLDRICK, B. & HIRSCH, J. (1963). A technique for quantitative recovery of lipids from chromatoplates. *J. Lipid Res.* **4**, 482–483.

GONZALEZ-JIMINEZ, E. & BLAXTER, K. L. (1962). The metabolism and thermal regulation of calves in the first month of life. *Br. J. Nutr.* **16**, 199–212.

HALES, J. R. S., FINDLAY, J. D. & ROBERTSHAW, D. (1968). Evaporative heat loss mechanisms of the newborn calf, *Bos taurus*. *Br. vet. J.* **124**, 83–88.

HEIM, T. & HULL, D. (1966). The blood flow and oxygen consumption of brown adipose tissue in the new-born rabbit. *J. Physiol.* **186**, 42–55.

HILL, JUNE R. (1959). The oxygen consumption of new-born and adult mammals. Its dependence on the oxygen tension in the inspired air and on the environmental temperature. *J. Physiol.* **149**, 346–373.

JANSKY, L. & HART, J. S. (1963). Participation of skeletal muscle and kidney during non-shivering thermogenesis in cold-acclimated rats. *Can. J. Biochem. Physiol.* **41**, 953–964.

MCLEAN, J. A. (1963). Measurement of cutaneous moisture vaporization from cattle by ventilated capsules. *J. Physiol.* **167**, 417–426.

MOORE, J. H. & WILLIAMS, D. L. (1963). The influence of dietary fat on the liver lipids of the rabbit. *Can. J. Biochem. Physiol.* **41**, 1821–1835.

MOORE, J. H. & WILLIAMS, D. L. (1964). Some observations on the specificity of phospholipase A. *Biochim. biophys. Acta* **84**, 41–54.

MOORE, R. E. & UNDERWOOD, MARY C. (1963). The thermogenic effects of noradrenaline in new-born and infant kittens and other small mammals. A possible hormonal mechanism in the control of heat production. *J. Physiol.* **168**, 290–317.

MOUNT, L. E. (1959). The metabolic rate of the new-born pig in relation to environmental temperature and to age. *J. Physiol.* **147**, 333–345.

NELSON, G. T. & FREEMAN, N. K. (1959). Serum phospholipid analysis by chromatography and infra-red spectrophotometry. *J. biol. Chem.* **234**, 1375–1380.

RIZACK, M. A. (1961). An epinephrine-sensitive lipolytic activity in adipose tissue. *J. biol. Chem.* **236**, 657–662.

SCOPES, J. W. & TIZARD, J. P. M. (1963). The effect of intravenous noradrenaline on the oxygen consumption of new-born mammals. *J. Physiol.* **165**, 305–326.

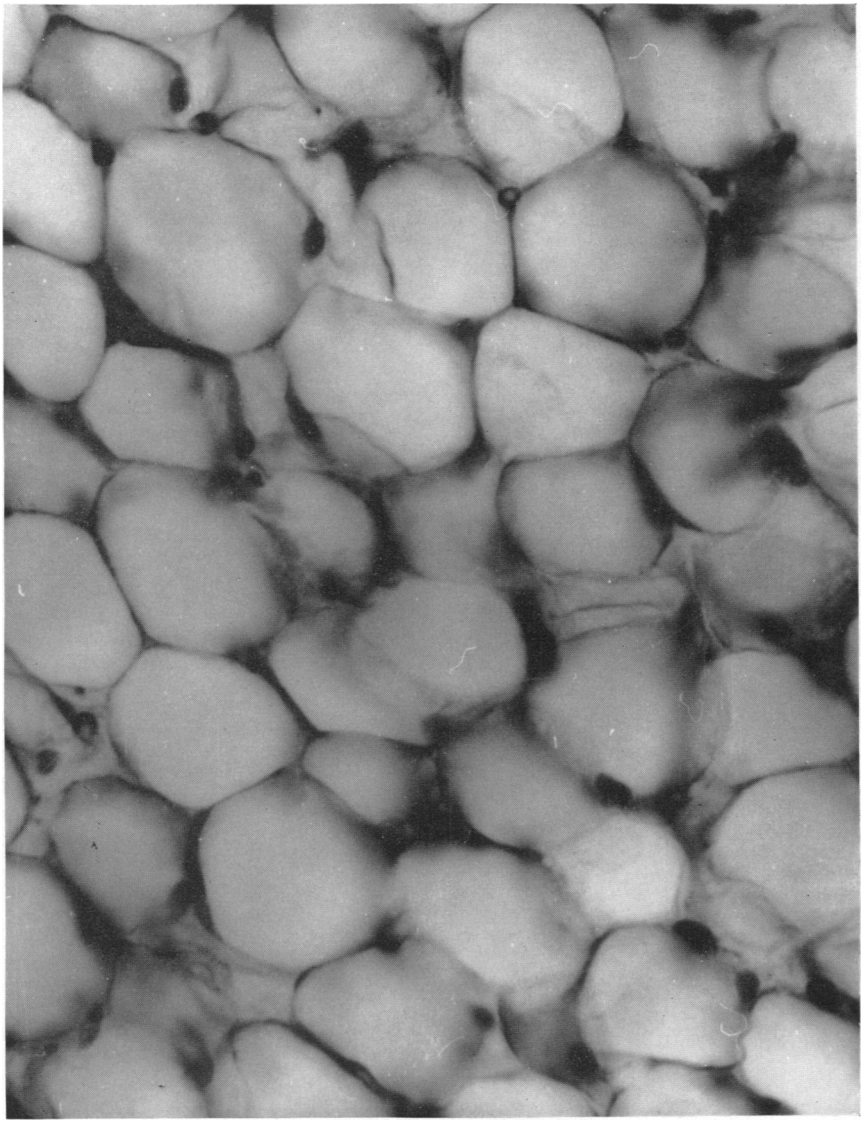
STOFFEL, W., CHU, FLORENCE & AHRENS, E. H. (1959). Analysis of long-chain fatty acids by gas-liquid chromatography. *Analyt. Chem.* **31**, 307–308.

TAYLOR, P. M. (1960). Oxygen consumption in new-born rats. *J. Physiol.* **154**, 153–168.

WREN, J. J. & SZCZEPANOWSKA, ANNA D. (1964). Chromatography of lipids in presence of an antioxidant 4-methyl-2, 6-di-tert-butyl phenol. *J. Chromat.* **14**, 405–410.

EXPLANATION OF PLATE

Photomicrograph of perirenal adipose tissue of a 6-day-old calf.



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