There was similarly no appreciable difference in response between normal and actinomycin D treated animals to the intravenous injection of 200 μg methoxamine.

Discussion. In view of the evidence that actinomycin D does not seriously interfere with the function of the phasic contractile elements, it appears that the blood pressure depression may be a reflection of a reduced arterial tone. Any attempt to explain the mechanism of this effect would be premature. To suggest that the cellular potassium-protein system participates in this response requires first of all proof that actinomycin D suppresses synthesis of muscle cell protein. Studies are now underway designed to resolve this question.

Summary. Actinomycin D causes a fall in blood pressure of normotensive and renal hypertensive rats; the reduction of pressure is proportional to the dosage. Giving prednisolone did not restore the pressure but hormonal activity is not blocked since prednisolone protects against a lethal dose of actinomycin D given to adrenalectomized rats. Vigorous arterial pressor response to norepinephrine and methoxamine limits any peripheral arterial effect to tonic rather than phasic contraction.

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Lipoprotein Lipase Activity (LLA) in Adipose, Muscle and Aortic Tissue from Rats of Different Age and in Human Subcutaneous Adipose Tissue.* (30298)

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The presence of lipoprotein lipase activity (LLA) in the adipose tissue of rats, rabbits and chickens is well established(1,2,3) but its presence in significant quantities in human adipose tissue has been questioned(4,5).

Angervall(4) found that lipoprotein lipase activity was considerably greater in chicken than in hen adipose tissue. Using a modification of the method described by Cherkes and Gordon(6), Chlouverakis(7) found that LLA in the adipose tissue of rats 4-5 weeks old was significantly greater than that of rats more than 5 months old. In interpreting this finding, however, it must be borne in mind that the protein/lipid ratio of young and old rat adipose tissue may differ(8).

This possibility was investigated by measuring the LLA in the adipose tissue of 2 groups of rats, one consisting of animals 4 to 6 weeks old and the other of much older animals (5 to 8 months), and expressing the results as LLA per unit of tissue wet weight and per unit of tissue protein. Other possible explanations for the apparent enzyme change associated with the ageing process, namely, a redistribution of the enzyme and its relationship to growth hormone, were also investigated.

Finally, in view of the conflicting reports regarding the enzyme activity in human adipose tissue, the LLA of subcutaneous fat was determined in material taken at biopsy.

Material and methods. Tissues. Male Wistar rats fed on a standard diet (Pillsbury's Thompson Diet Rat Cube, Birmingham, England), were used in all the experiments. "Young" rats were 4-6 weeks old and "old" rats were aged 5-8 months. Animals were killed by decapitation, adipose tissue was ob-

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tained from the epididymal fat body, muscle tissue from the diaphragm and the whole aorta down to its division to the iliac arteries.

Human subcutaneous adipose tissue was obtained from the buttocks of 7 male patients attending the Endocrine Clinic of this hospital for oestrogen implantation and from one obese middle-aged woman. About 1 g of tissue was obtained following local anaesthesia with lignocaine and immediately after excision the tissue was placed in chilled buffer, thoroughly cleansed of blood and fibrous tissue, and its LLA assayed.

Enzyme assay. Lipoprotein lipase activity was assayed by the method described by Cherkes and Gordon(6) in which the tissue is first incubated in a heparin-containing buffer and the lipase released into the incubating medium is then assayed by its lipolytic effect on lipoprotein-triglycerides. Pieces of tissue weighing between 80 and 120 mg (apart from the aortic tissue which weighed between 15 and 50 mg) were placed in tubes containing one ml of Krebs-Ringer bicarbonate buffer(9) to which sodium-heparin was added to a final concentration of 50 μg (5.66 units) per ml.

In this medium tissues were incubated at 37°C on a shaking incubator (Gallenkamp and Co., England) for 50 minutes to allow the lipoprotein lipase to escape from the tissue into the medium(6). Then a 0.5 ml aliquot of the medium in which the tissues had been incubated was mixed in a glass-stoppered tube with an equal volume of a substrate solution prepared by mixing 8 parts of a 10% solution of crystalline bovine albumin (Armour) adjusted to pH 8.8 with 0.5 M NH_4OH, 1 part of 5% cottonseed oil emulsion (infonutrol) and 1 part of fresh human plasma and incubating the mixture for 30 minutes at 37°C. The substrate-medium mixture was incubated at 37°C in a similar manner for a period of one hour after which the enzyme reaction was stopped by adding 5 ml of Dole's extraction mixture(10). The extent of lipolysis was determined by the difference in NEFA content between tubes containing medium from experimental and control flasks, the latter containing no tissue. NEFA content was measured by the method of Dole (10) using a modified titration procedure (11). Results were expressed in units of lipoprotein lipase, one unit representing one μEq of NEFA liberated during the one-hour incubation.

Tissue protein determination. Following incubation in the heparin-containing medium, the pieces of tissue were homogenized in Potter homogenizing tubes containing 5 ml of 10% (w/v) trichloroacetic acid (TCA). After centrifugation the protein was measured in the precipitate by a modification(12) of Waddel's method(13) adapted as follows:

The TCA precipitate was extracted into 5 ml of N/10 NaOH and after centrifugation the protein of the clear supernatant was reprecipitated with an equal volume of 10% (w/v) TCA. This procedure of protein purification was repeated once more and after taking up the final precipitate in NaOH solution, an aliquot of this was diluted with distilled water to a final concentration of 0.005 N and read in a Unicam spectrophotometer at 225 and 215 mμ. This ultraviolet spectrophotometric method of protein determination has been shown(13) to be in good agreement with the Kjeldahl method.

Effect of growth hormone on the adipose tissue LLA. The effect of growth hormone *in vivo* was studied by injecting "old" rats intraperitoneally with 5 mg human growth hormone in 1 ml N/100 NaOH daily for 2 days and finally 3 hours before the animal was killed on the third day. "Control" animals of the same age were injected intraperitoneally with 1 ml of N/100 NaOH.

The *in vitro* effect of human growth hormone was tested by adding 100 μg of the hormone per ml of the heparin-containing Krebs-Ringer bicarbonate incubating medium.

Results. Table I shows the LLA of adipose, muscle and aortic tissue from young and old rats.

When the LLA was expressed in enzyme units per gram of wet weight of tissue only the LLA of the adipose tissue differed between the two groups of animals, that of the young animals being almost 4 times that of the old animals ($p<0.0002$). The LLA of the two other tissues tested was virtually the same.

TABLE I. Adipose, Muscle and Aortic Tissue Lipase Activity (LLA) (Mean ± S.E. of Mean) in "Young" and "Old" Rats. Enzyme activity is expressed in units per gram of wet weight of tissue and per 100 mg of tissue protein.

Tissue	LLA per g wet wt		LLA per 100 mg tissue protein	
	Young rats	Old rats	Young rats	Old rats
Adipose tissue	15.8 ±2.5 (13)	4.2 ±1.3 (13)	56.0 ±12.0 (13)	25.4 ± 5.5 (13)
Diaphragm	8.7 ± .9 (17)	8.1 ± .9 (17)	5.3 ± .5 (17)	5.2 ± .6 (17)
Aorta	2.7 ± .9 (12)	3.0 ± .8 (11)	3.5 ± 1.7 (12)	3.9 ± 1.1 (11)

Numbers in parentheses indicate No. of animals.

When LLA was expressed not in relation to the wet weight of the tissue but referred to 100 mg of tissue protein, the adipose tissue LLA was still greater in the young animals although only twice that in the old animal ($p<0.05$). There was no difference in the muscular and aortic tissue LLA between the two groups of rats.

Human growth hormone whether injected into the animal or added to the incubation medium at a concentration of 100 μg/ml did not affect the LLA of the tissues tested.

The LLA of adipose tissue from 6 non-fasting adult human subjects (5 males and one female) was found to be 1.2 ± 0.4 unit/g (mean ± S.E.M.) with a range of 0.0 to 2.3 units/g and the enzyme activity from 3 fasting human subjects (all males) was 0.5 ± 0.1 unit/g (mean ± S.E.M.) the difference between these 2 values not being of statistical significance ($p = 0.2$).

Discussion. The above results confirm that the adipose tissue lipoprotein lipase activity (LLA) of rats is influenced by age. A similar age effect has been reported by Angervall in the chicken(4).

The fact that the difference, although smaller, was still significant when the protein content of the tissue was taken into account suggests that this age effect on the LLA is a specific one occurring in addition to a similar effect of age on the protein/lipid ratio of the tissue.

The mechanism of this effect and its physiological significance is not clear. In view of the failure of human growth hormone to affect the LLA of adipose tissue either *in vivo* or *in vitro* it is unlikely that this hormone is the mediating mechanism. Similarly a redistribution of the enzyme occurring as age advances is not likely, in view of the very similar values of LLA in other tissues tested from the two groups of animals.

The existing experimental evidence favors the view that lipoprotein lipase participates in the formation of fat depots by facilitating the transport of fat from the blood into the adipose tissue(3,14). Thus, the absolute fall of LLA in the adipose tissue of old animals would be accompanied by a parallel fall in the rate of accumulation of depot fat. In a "steady state" this would imply a slower mobilization of fat from the depots and this has been supported by the work of Benjamin *et al*(8), Jelinkóva-Tenóróva and Hruza(15), and Hruza and Jelinkóva(16). Work still in progress(17) gives further support to the hypothesis of a faster turnover of fat to and from the adipose tissue of young rats compared with older animals.

An alternative explanation for these findings would be that there is no difference with age in the absolute amount of the LLA of adipose tissue but that, under the influence of heparin, the enzyme can be released more easily from the adipose tissue of young rats. If this were the case one might have expected a similar effect of heparin on other tissues as well.

The findings reported here, regarding the lipoprotein enzyme activity (LLA) of human adipose tissue, are in keeping with those of Engelberg(5) Angervall(6), and with those concerning the enzyme activity of human mammary adipose tissue reported previously from this laboratory(7). In this light it is difficult to explain the findings of Stern, Iacono and Mueller(18) who reported appreciable LLA in human adipose tissue. The latter workers discussing the failure of other investigators to reveal significant amounts of this enzyme in human adipose tissue sug-

gested that this failure might have been due to the presence of blood, containing enzyme inhibitors. This, however, is unlikely to be the case in the experiments reported here as macroscopic blood whenever present was carefully removed. Differences in methodology are more likely to contribute to the conflicting results from various laboratories but, whatever the cause, the existence of lipoprotein lipase in the adipose tissue of man in physiologically significant quantities still remains an open question.

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Lysosomal Enzymes in Bruised Tissue.* (30299)

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A great amount of literature concerning biochemical and physiological anomalies in disease and following injury of various types has appeared in the past few years. Investigations into the nature of the bruising phenomenon, however, have been the prime interest of Hamdy *et al,* who studied the biochemical changes resulting from blunt injury (1,2), the effect of ascorbate on healing(3), developed a method for estimating the age of a bruise(4) and measured changes in tissue conductivity and permeability as a result of bruising(4). In addition, Hamdy *et al* observed increased proteolytic activity, optimally active at pH 8.0, in bruised tissue and an accelerated healing upon injecting streptokinase or trypsin into bruises(5). Rate of healing was also affected by such factors as severity of contusion, environmental temperature, previous bruise history, and passive transfusion of blood from a previously bruised animal to one not previously bruised(2,3,6,7).

These changes, as well as investigations into other types of traumas(8-13), suggested the presence of high levels of hydrolytic activity as a result of trauma. The massive proteolysis, necrosis and phagocytic invasion observed by Schmittle and Rogers(14) during pathological and histochemical studies on experimentally inflicted poultry bruises further implicated the function of hydrolytic enzymes. Accordingly, the present study deals with a relatively recently recognized cellular organelle, the lysosome, which is known to contain an array of hydrolytic enzymes(15).

The main objectives of this study were to determine the effects of bruising on the following lysosomal(15) enzymes (β-galactosid-

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