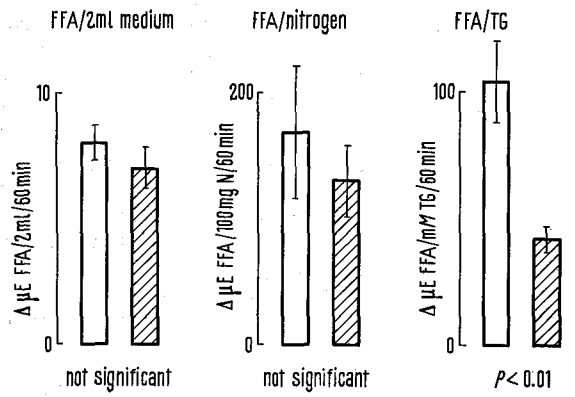
triglyceride[10] content usually carried out to determine the approximate amount of isolated fat cells. The results obtained showed that an approximately equal number of fat cells was obtained from the isolated fatty tissue in older rats (nitrogen in 2-month-old rats 0.237 mgN/ml ± 0.029, in 14-month-old rats 0.266 mgN/ml ± 0.0154; P = not significant) with a higher triglyceride (TG) content (in 2-month-old rats 39 μM/ml ± 4.11, in 14-month-old rats 84 μM/ml ± 3.52; $P < 0.01$). This ratio of nitrogen to triglycerides is in accord with data on the general composition of fatty tissue showing that there was a decrease in nucleic acids and a relative increase in the amount of triglycerides/U weight in older rats (μg P_{NA}/100 mg in 2 month: 8 ± 0.477 month: 5.34 ± 0.578; $P < 0.05$; μM TG/100 mg in 2-month-old rats: 88.3 ± 9.5; in 14-month-old rats: 107 ± 6.22; P = not significant).

The increment of free fatty acids found in the medium was then evaluated both in relation to the volume of the incubation medium and to the nitrogen and triglyceride content (Figure). This showed in the first place that the age difference in the amount of free fatty acids liberated is only significant in relation to triglyceride values. When the amount of liberated free fatty acids was related to nitrogen content or total volume of the medium, it certainly had a falling trend with increasing age, but the differences are not statistically significant.

Yet another experiment was made with the same set-up, using noradrenaline concentrations of 2.5 μg/ml as lipomobilizing substance. Exactly the same relationships were found, i.e. the amount of free fatty acids liberated calculated to the volume of the medium and to nitrogen content is not significantly lower in 14-month-old rats, whereas when calculated to triglyceride content lipolysis was strikingly lower in older rats ($P < 0.001$).

It would seem that the decreased lipolysis of fatty tissue in older rats is caused by alterations in the character of fatty tissue itself. The triglyceride content is increased in the fat cells so that the relative amount of active protoplasm is decreased both in relation to volume and weight; this means a relative decrease in the enzymatic equipment of the cell[6]. This is not a case of absolute decreased response (activity) of hormone-sensitive lipase which is responsible for lipolysis. Aging alters the morphological character of fatty tissue and this is the basis of the altered metabolic activity found in older rats. It is, therefore, necessary, and we consider this to be the main conclusion from our present work, to revise the other findings relating to the metabolism of fatty tissue in rats of different age from this point of view.

In addition, the question remains of what is the immediate cause of the accumulation of triglycerides in fat cells with increasing age. Since the incorporation of acetate and palmitate into triglycerides of fatty tissue is decreased[5], there is no question of an increased synthesis of triglycerides with age. We cannot, of course, exclude a long-continued but not significant decrease in lipolysis in the course of aging being a factor. One piece of evidence for this hypothesis is perhaps the finding that lipolysis of isolated fat cells in relation to the same amount of nitrogen as metabolically active unit is slightly decreased in older rats (even if non-significantly) as compared with young rats.



Free fatty acids (FFA) released by free fat cells in response to epinephrine 2.5 g/ml in relation to the volume of the medium, to the nitrogen content and to the triglyceride (TG) content of isolated fat cells. ■ rats aged 2 months, ▨ 14 months.

Zusammenfassung. Die Freigabe unesterifizierter Fettsäuren in ein Medium aus isolierten Fettgewebezellen älterer Ratten (14 Monate) ist im Vergleich mit jüngeren Ratten (2 Monate) kleiner im Verhältnis zu den Triglyzeriden und nur unbedeutend in der Relation zum Stickstoff oder zum Volumen des Mediums.

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Pressor Response to Angiotensin I During Cardio-Pulmonary Bypass

The lungs must play an important role in the activation of the decapeptide angiotensin I since the administration of a given amount into the right heart gives rise to twice as much musculotropic[1,2] and pressor[3,4] activity as a similar dose introduced into the left heart. Conversion into the octapeptide angiotensin II in whole blood circulating extracorporeally was reported to be of only 14% after 15 sec of contact[1] and is therefore too slow to be physiologically significant. It is of importance to determine whether the lungs are the exclusive site of activation for angiotensin I; such a possibility would force a revision of current concepts[5,6] of the intrarenal role of juxtaglomerular renin by making it impossible for the kidney to generate angiotensin II within its own circulation. This question of the exact contribution of the lung could best be approached by studying angiotensin I activation following total removal of that organ. In 2 dogs, the systemic pressor responses to intra-arterially injected angiotensin I

was examined before and after excluding the lungs from the circulation by means of a complete cardio-pulmonary bypass.

Methods. 2 female mongrel dogs weighing 22 and 18 kg, respectively, were anaesthetized with i.v. pentobarbital. Mechanical ventilation was provided through an endotracheal tube. Blood pressure in the lumbar aorta was monitored through a Statham transducer connected to a tube inserted into the right femoral artery. The dogs were placed in the supine position. The chest wall was opened through a transverse bilateral anterior thoracotomy. Heparin, 3 mg/kg, was injected i.v. A catheter was introduced into the left atrium to allow for a series of injections, first of angiotensin I, then of angiotensin II. Total cardio-pulmonary bypass was established by cannulation of both vena cavae and of the left femoral artery. A Lillehei-DeWall bubble oxygenator was primed with 2.6 l of homologous whole blood drawn in heparin (60 mg/l) on the eve of surgery and preserved at + 4 °C overnight. The flow rate was adjusted at 1.5 l/m² · min (1.350 and 1.150 for dogs 1 and 2) by means of a totally occlusive roll up pump. Transit time through the oxygenator was 2 min. A cardiotomy sucker was placed into the main pulmonary artery through the right atrium to aspirate all blood from the right heart. Each pulmonary hilus was then securely clamped by means of a tourniquet in order to exclude all systemic circulation to the lungs. Injections of angiotensin I, then of angiotensin II, were made into the arterial line 10 cm from its entry into the left femoral artery.

Angiotensin II was CIBA Hypertensin. Angiotensin I was synthetized by Dr. M. BUMPUS, using the solid phase method. We have expressed the amounts of injected angiotensin I in terms of angiotensin II equivalents instead of indicating the actual weight which was 3 times higher. The systemic pressor activity was assayed by determining the dose of each peptide required to increase diastolic aortic pressure by 20 mmHg. Graded doses were separated by an interval of 5–10 min, doubling the previous dose each time until a pressor peak of at least 25 mmHg was obtained. The log-dose was linearly related to the pressor response and the exact amount that could induce a 20 mmHg rise was calculated by bracketing.

Results. Left-heart injections of angiotensin I were half as potent as those of angiotensin II (Table). When compared to the responses to angiotensin II, the decapeptide pressor peaks started about 5 sec later and reached their maximum about 10 sec later. These phenomena have been shown in rats[4] and suggest that the first portion of the angiotensin I response is due to relatively slow blood and/or tissue activation, whereas the main portion of the peak occurs upon recirculation of the pulmonary activated peptide.

Femoral artery injections during the bypass were followed by an immediate pressor response to both types of angiotensins. No secondary peak occurred 2 min after each injection, as would be expected if the peptides had survived passage through the systemic vascular beds of the dogs and through the oxygenator. This absence of recirculation is easily explained, considering that (a) removal of both peptides by canine systemic beds exceeds 66%[1,2,7,8] and (b) the third which escaped into the venous return was diluted for 2 min into 2.6 l of angiotensinase-containing blood filling the oxygenator circuit. During the bypass procedure, diastolic aortic pressure fell from 90–70 mmHg and the systolic pulse disappeared. The sensitivity to angiotensin I decreased almost 60 times and the pressor curve was slightly delayed as for left-heart injections; the sensitivity to angiotensin II diminished only 20 times.

Discussion. The first pertinent observation emerging from these experiments is that during exclusion of the lungs from the circulation, the systemic pressor response to intra-arterially administered angiotensin I is not entirely abolished. Activation must have taken place at or near arteriolar receptor sites and it follows that the lungs are not the only site of conversion in the body. This conclusion is further supported by the fact that homogenates of other organs contain as much convertase activity as those of lungs[9].

The second salient observation is that the bypass procedure reduced the response to angiotensin I almost 3 times more than that to angiotensin II. From the present data, it would seem that $^2/_3$ of the overall activation occur in the pulmonary system: this figure must not be taken as more than suggestive, however, since the present technique had an error coefficient of about 20%, only 2 animals were tested, and infusions were not carried out[10].

Résumé. La réponse pressive systémique à des injections intra-artérielles d'angiotensine I et II a été étudiée chez deux chiens avant et après avoir totalement exclu les poumons de la circulation. La réactivité à l'angiotensine I diminua presque 3 fois plus que celle à l'angiotensine II durant le shunt cardio-pulmonaire. Il apparaît donc que la circulation pulmonaire est un site important mais non exclusif de conversion de l'angiotensine I.

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	Left atrium (before bypass)	Femoral artery (during bypass)	Femoral/atrium dose ratio
Angiotensin II	9	200	22
ng/kg	11	230	21
Angiotensin I	19	1100	58
ng/kg	21	1150	55

Intra-arterial pressor dose required to raise diastolic pressure by 20 mmHg. Doses indicated for angiotensin I represent angiotensin II equivalents. Upper and lower figures from dogs 1 and 2, respectively.

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