Rf que le 21 monoacétate de 5β-prégnane 17α, 21-diol 3, 11, 20-trione (V) dans les systèmes toluène/propanediol et benzène/formamide.

La Figure montre les étapes suivies pour l'identification du stéroïde isolé.

Propriétés physico-chimiques. Le composé isolé des urines donne une réaction positive au bleu de tétrazolium et au réactif de Porter et Silber, ne présente pas d'absorption à 254 mμ, et il n'est pas fluorescent en milieu sodique à la lumière de Wood et, après oxydation bismuthique ou chromique, il se transforme en un 17-cétostéroïde.

Evaluation quantitative. En utilisant la micro-réaction au bleu de tétrazolium, nous avons évalué à 150 $\mu g^0/_{00}$ la quantité de dihydrocortisol présent dans l'urine.

Discussion. On sait que le cortisol est converti dans l'organisme essentiellement en dérivés tétra et hexahydrodérivés: 5β-prégnane 3α, 11β, 17α, 21-tétrol 20-one (tetrahydrocortisol) 5α-prégnane 3α, 11β, 17α, 21-tétrol 20-one (allo-tétrahydrocortisol); 5β-prégnane 3α, 17α, 21-triol 11, 20-dione (tétrahydrocortisone); 5β-prégnane 3α, 11β, 17α, 20α, 21-pental (cortol) et ses isomères en 5α et en 20β; 5β-prégnane 3α, 17α, 20α, 21-tétrol 11-one (cortolone) et son isomère 20β[3;4]. Parmi les composés mineurs qui ont été isolés citons: le prégna 4-ène 11β, 17α, 20α, 21-tétrol 3-one (composé epiE de Reichstein)[5], le prégna 4-ène 11β, 17α, 20β, 21-tétrol 3-one (composé E de Reichstein)[6], le prégna 4-ène 17α, 20β, 21-triol 3, 11-dione (composé U de Reichstein)[7].

SCHNEIDER[8] a également isolé des urines humaines le 5β-prégnane 17α, 21-diol 3, 11, 20-trione (dihydrocortisone); tous ces métabolites sont des dihydrodérivés qui résultent de la réduction soit du carbonile en C_{20}, soit de la double liaison en C_{4-5}; en revanche, le 5β-prégnane 11β, 17α, 21-triol 3, 20-dione (dihydrocortisol) n'a pas été identifié dans l'urine humaine à notre connaissance.

En montrant que le corticostéroïde que nous avons isolé a dans six systèmes différents les mêmes Rf que le dihydrocortisol, que son acétate a le même Rf que l'acétate de dihydrocortisol dans deux systèmes différents, et qu'après oxydation bismuthique ou chromique il se transforme respectivement en 5β-androstane 11β-ol 3, 17-dione et 5β-androstane 3, 11, 17-trione, nous avons apporté la preuve que ce corticostéroïde est le 5β-prégnane 11β, 17α, 21-triol 3, 20-dione.

Summary. 5β-pregnane 11β, 17α, 21-triol 3, 20-dione (dihydrocortisol) has been isolated and identified in the urine of patients with feminizing adrenal cortical cancer. This steroid showed the same Rf as synthetic dihydrocortisol in six different chromatographic systems. After acetylation, its Rf was the same as that of 21 monoacetate of dihydrocortisol. After bismuthic or chromic oxydation, it had the same chromatographic Rf as 5β-androstane 11β-ol 3, 17-dione and 5β-androstane 3, 11, 17-trione respectively. After chromic oxydation of its acetate, the Rf was the same as that of 21 monoacetate of cortisone.

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Metabolism of the Normal Cardiovascular Wall. 1. Utilization of Glucose by Arteries and Veins

In order to establish the metabolic pattern of the normal vascular wall, a series of studies are being undertaken to determine the role, fate and relative significance of the principal substances involved in the metabolic process.

This communication presents the results of the first group of experiments concerning the metabolism of glucose and the *qualitative* as well as *quantitative* determinations of its intermediate metabolites in normal arteries and veins of the dog.

Materials and Method. Uniformly ^{14}C-labeled glucose was obtained from the Radiochemical Centre, Amersham (England). The radioactive material was diluted with inert substrate to give a specific activity of 8 μc/mg. Adult, male, mongrel dogs, weighing about 15 kg were anaesthetised intraperitoneally with nembutal. The *popliteal* and one of the *colic* arteries and their accompanying veins were dissected out. Before removal of the vessels, the adventitia was carefully stripped off, after which they were split longitudinally into strips weighing about 50 mg and placed into 0.6 ml of ice-chilled Krebs-Ringer phosphate medium at pH 7.4 in Warburg flasks. The glucose concentration was 0.3% and each flask contained 14 μc of the radioactive substance. A small roll of filter paper soaked with 0.5 ml of 30% NaOH was placed in the centre well of each, and the flasks were then attached to the manometers for an incubation period of 120 min at 37°C, in oxygen.

Similar experiments were conducted under anaerobic conditions, but in these the glucose was placed in the side-arm of the Warburg flask and added to the medium only after equilibration with nitrogen. The gas was passed over heated copper wire to remove all traces of oxygen.

Radioactive CO_2 production was estimated after the method of VILLEE and HASTINGS[1]. The techniques used in the preparation of the acqueous alcohol extracts for mono- and bidimensional radio paperchromatography were those described by BELOFF-CHAIN et al.[2]; and the quantitative determinations were greatly facilitated with the automatic scanning device designed by FRANK[3]. The solvents used for the bidimensional chromatograms are given in the Figure. Monodimensional chromatograms,

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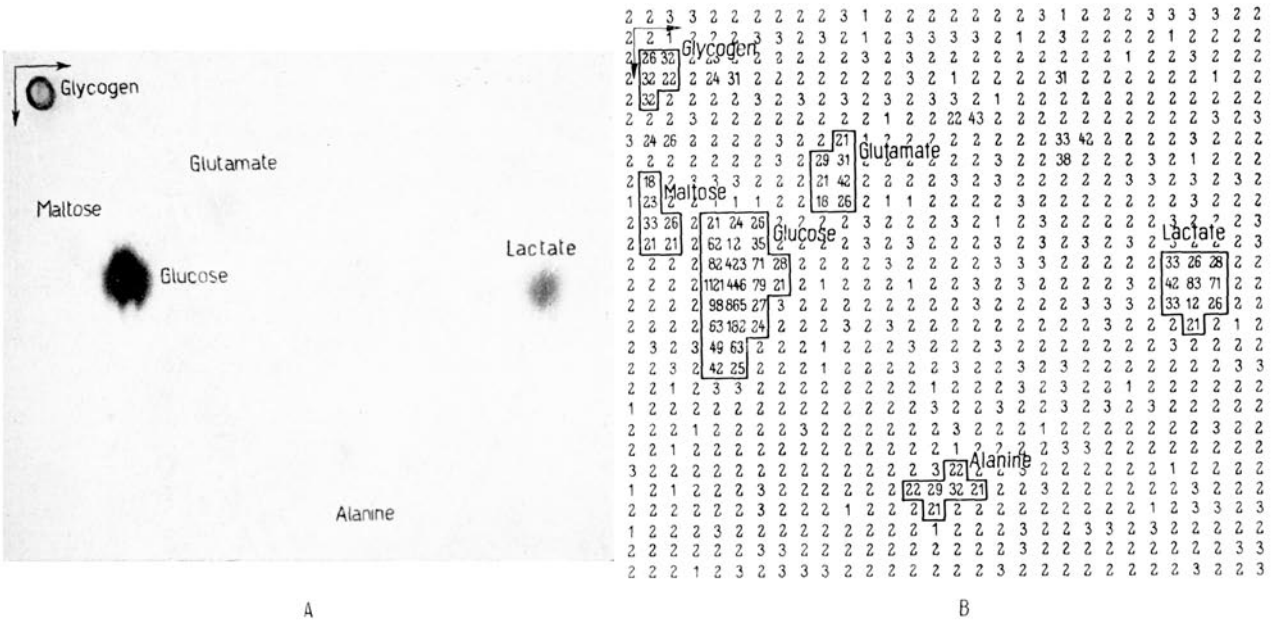

(A) Radioautograph of a bidimensional chromatogram - acqueous alcohol extract of popliteal artery incubated with uniformly ^{14}C-labeled glucose. (B) Corresponding number map obtained from automatic scanner-Traces of some Krebs cycle intermediates are evident between the glutamate and lactate spots. (Chromatogram developed in → 75 ml sec - butanol, 15 ml of 85% formic acid, 15 ml water; ↓ 80 ml phenol, 20 ml water and 1 ml ammonia $d = 0.910$.)

Tab. I. The fate of glucose in the popliteal artery of the dog

CO_2	Lactate	Glycogen	Maltose	Glutamate	Alanine	Insoluble residue	Total glucose ^{14}C metabolized	Unconverted glucose ^{14}C remaining in tissue	Total uptake
1.4 ± 0.1	55.6 ± 3.2	2.5 ± 0.6	2.7 ± 0.6	1.3 ± 0.7	1.8 ± 0.2	0.8 ± 0.1	69.1 ± 4.8	46.5 ± 2.1	115.6 ± 3.6

Results are expressed as μg glucose converted per 50 mg of tissue (wet weight) after 120 min incubation at 37°C in O_2 in 0.6 ml of Krebs-Ringer phosphate buffered medium pH 7.4; glucose concentration 0.3%; total radioactivity of uniformly ^{14}C-labeled glucose, 14 μc per flask; mean values of 16 experiments ± S.E.

for the measurement of lactic acid and glucose, were developed, respectively, in (a) 40 ml normal butanol, 11 ml acetic acid and 25 ml water; and (b) 80 ml tertiary butanol, 4 g picric acid and 20 ml water.

To prevent loss of the lactic acid by evaporation, the chromatograms were exposed to ammonia vapour, which converts the free acid into ammonia salt.

After extraction of the tissues, all of the insoluble residues were collected, and half of the total amount was transferred to planchets and counted. The other half was used for fat extractions (petroleum ether, b.p. 40-70°C) and the residue from the evaporated petroleum ether was transferred to a planchet and counted.

Results. Under the experimental conditions described, the pattern of glucose metabolism in the popliteal artery can be seen from the Figure, and the relevant quantitative estimations are shown in Table I. The principal metabolites formed were lactate, glycogen and oligosaccharides, glutamate, alanine, CO_2 and traces of the tricarboxylic acid cycle. The mean oxygen consumption was 0.14 ± 0.01 ml/mg of tissue wet weight/h. The presence or absence of glucose in the media revealed no noticeable difference in the oxygen uptake.

The quantitative data given in Table I show that the popliteal artery has a high glycolytic activity. In fact, some 80% of the glucose metabolized was accounted for as lactate, as reported also by other investigators[4,5]. In our experiments, this value includes the lactic acid in both the medium and the extract, found in a proportion of 10:1.

The artery is known to store relatively large amounts of glycogen[6]. Our studies reveal a considerable formation, about 8%, of glycogen and maltose. As far as glycolytic activity is concerned, the arterial wall is similar to striated muscle[2], although the normal utilization of glucose in the latter is three or more times higher. However, the formation of amino acids from glucose varies greatly in the two types of tissue. Whereas in striated muscle only traces of amino acids have been found to be formed[2], the present investigation reveals that the arterial wall converts about 10% of the radioactive glucose into amino acids (glutamate 6.3% and alanine 2.6%). In this respect, the artery approximates more closely the results found in brain[7,8].

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The formation of CO_2 from radioactive glucose represents only 2% of the total glucose metabolized, and its relative specific activity is low in comparison to that found in other tissues studied[9,10].

The total glucose metabolized, calculated as the sum of all the fractions measured, was about 4%. Total uptake, i.e., glucose metabolized plus glucose unchanged in the tissue, was 6.4%. The composition of the radioactive content of the insoluble residues collected was determined and was found, in small part, to consist of lipides. Exact quantitative estimation of these fats is presently being done.

Under anaerobic conditions, the only measurable metabolite formed from glucose in the popliteal artery was lactic acid, as has also been demonstrated in striated muscle[11]. The mean value of lactic acid production in 8 experiments was 64.7 ± 10.6 mg/50 mg of tissue (wet weight). Comparison between aerobic and anaerobic glycolysis (Table II) shows an apparent increase under anaerobic conditions, which has also been reported by KIRK[4]. Statistical analysis of our results shows this difference not to be significant, indicating apparent *absence of the Pasteur effect* in this tissue.

The qualitative pattern of ^{14}C-labeled glucose in the popliteal vein and in the colic artery and vein was similar to that found in the popliteal artery. The quantitative values of the intermediate metabolites, however, varied considerably, both between the arteries and their corresponding veins and between the abdominal and the limb vessels. The metabolic activity of the popliteal vein was less than half, and often only 20%, of that found in the corresponding artery. This applies mainly to the CO_2 and lactic acid production, as the other metabolites were present in amounts considered too small for accurate estimation. In the colic arteries, glucose metabolism was observed to be some 20% higher than in the popliteal arteries, and this held true for all the intermediate metabolites mentioned in Table I. In the colic vein, results indicate a metabolic activity about 40% lower than in the corresponding artery, considerably less of a difference than that observed between the popliteal artery and vein.

Tab. II. Comparison of lactate production under aerobic and anaerobic conditions

O_2	N_2
55.4 ± 5.9	64.7 ± 10.6[a]

[a] Difference not statistically significant. Results expressed as μg glucose converted per 50 mg of tissue (wet weight).

The disparity in the utilization and metabolism of glucose between the arteries and veins studied can be attributed to the difference in their histological structure, particularly as regards the larger proportion of smooth muscle fibres present in the artery. The reasons for the quantitative difference between the abdominal and limb vessels, however, is not immediately apparent. Their diversity in histological composition alone could not account for it, so we probably have to look to their dissimilarity in function and anatomic location for an explanation. The possibility should be kept in mind, however, that this difference might be less marked under normal conditions. A general anaesthetic, irrelevant of the agent, does produce a marked peripheral vasodilatation and a corresponding central vasoconstriction[12–14].

Riassunto. Il destino del glucosio generalmente mercato ^{14}C in diverse arterie e vene del cane é stato studiato con l'uso di una tecnica quantitativa di radiocromatografia su carta.

I risultati non solo mostrano che nella parete arteriosa ha luogo una notevole glicolisi aerobica ed anaerobica, ma anche chiaramente indicano che una percentuale relativamente alta di glucosio viene trasformata in amino acidi (probabilmente attraverso il ciclo degli tricarbossilici) ed in grassi.

Quantitativamente è stato osservato che il tessuto arterioso utilizza maggiore quantità di glucosio di quello venoso e che tale differenza è tanto maggiore quanto più i vasi sono periferici.

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Metabolism of the Normal Cardiovascular Wall. 2. The Pentose Phosphate Pathway

The breakdown of glucose in animal tissue is achieved mainly through the Embden-Meyerhof (glycolytic) scheme. Alternative routes of carbohydrate degradation, however, are known to exist in plants and in some microorganisms; but the actual presence and the role and significance of these routes in animal tissues have only recently been submitted to investigation.

The pentose phosphate pathway appears to be the most important of these lesser-known metabolic routes, with the extent of its utilization varying in different tissues[1–6]. It does not play a part in glucose metabolism in striated muscle under normal physiological conditions, whereas it

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