23 days of administration. Four rats sacrificed after a 6-day period showed easily recognized histologic changes typical of excessive vit. A intake.

Vit. A phenyl ether proved to be without effect in rats run for periods of 6 to 11 days.

The limitations of the method are as follows: (1) It can be applied only when large dosages are used. The syndrome is not produced by small amounts of pure products or by oils low in potency. (2) In the absence of fractures, adequate histologic sections of bony structures (knee joint) must be used for diagnostic purposes. (3) The histology must be interpreted by one familiar with the typical response.

Received July 18, 1951. P.S.E.B.M., 1951, v77.

Isolation of an Androgenic Compound from the Adrenal Venous Blood of Cows.* (18938)

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Although it has been thought that the adrenal gland may be capable of secreting androgenic compounds, the evidence presented has been inconclusive and other alternatives have seemed equally possible. Sayers(1) has suggested that such androgenic material may be the result of subsequent metabolism of the C_{21} steroids secreted by the adrenal cortex.

Besides 17-hydroxycorticosterone and corticosterone the presence of small quantities of other compounds in the adrenal effluent was indicated by previous work(2,3). The nature of these substances, however, was not determined.

In an attempt to obtain larger quantities of these compounds for identification, a special method was devised for the cannulation of the adrenal sinus of the cow. This has permitted the collection of large volumes of adrenal venous blood from cows stimulated with ACTH. At least 2 other compounds have been separated in addition to 17-hydroxycorticosterone(4). One of these fractions has now been demonstrated to have androgenic activity.

Experimental. Twenty liters of adrenal venous blood were collected from the adrenal sinuses of 3 cows which received an average of 600 mg ACTH intravenously during the blood collection. The animals also received intravenous infusions of heparin prior to the actual collection of blood and 5% glucose in saline during the 2-4 hour experiments. The blood was laked with an equal volume of H_2O and extracted with chloroform. The chloroform-extracted lipids were first partitioned between 70% ethanol and hexane and then between 30% methanol and hexane. The ketonic fraction of the 30% methanol-soluble lipids was acetylated and chromatographed on neutral aluminum oxide (Harshaw). Elution from the column was carried out with hexane-benzene, benzene, benzene-ether, ether-chloroform, and chloroform.

A fraction was eluted by the benzene-ether mixtures. This had an absorption maximum in the ultraviolet at 240 mμ and gave a positive Zimmerman reaction with maximum ab-

* This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

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TABLE I. Determination of Androgenic Activity by Chick Comb Inunction with Androstadiene, and Fraction Obtained from Adrenal Venous Blood.

Group	No. chicks	Treatment	Total dose, γ	Body wt (Bwt) Init.	Body wt (Bwt) Final	Comb wt mg	Comb wt % increase	Comb wt mg % Bwt	Comb wt % increase	Testes wt mg	Testes wt % diff.	Testes wt mg % Bwt
1	10	Ether oil	0	40	86	48	—	56	—	29	—	33
2	10	Androsterone	15	39	82	163	230	199	238	24	-17	29
3	10	Adrenal blood	1.6	40	70	64	33	91	63	20	-31	28
4	10	" "	16	40	78	90	88	115	105	22	-24	29

sorption at 520 mμ(5). The infrared spectrum indicated an α, β-unsaturated ketone structure and the presence of hydroxyl groups. Chromatography of the semicarbazones formed from this fraction suggests the presence of two compounds.

This fraction was assayed for androgenic activity by a modification of the chick comb growth method(6). Three-day-old White Leghorn cockerels were sorted as to weight into groups of 10 chicks per group. The steroids and extracts to be tested were dissolved in a mixture of 20% corn oil and 80% diethyl ether. Each chick received daily for 10 days, 0.02 ml of the respective solutions by application to the comb with a micropipette. The chicks were sacrificed 24 hours after the last inunction. Body, comb, and testes weights were determined, and the latter fixed in Bouin's fluid for histological evaluation. The results are indicated in Table I. It is evident that this fraction from adrenal venous blood was androgenic in nature.

A similar fractionation was carried out on peripheral blood from the same cows. No material showing the characteristics of the adrenal blood fraction was found.

Discussion. Masculinizing tumors of the adrenal gland associated with the excretion of large quantities of androgenic 17-ketosteroids have suggested that the adrenal gland might produce such compounds. These glands are functioning abnormally however, and cannot be used as an indication of normal function. Signs of masculinization have also followed ACTH therapy. Finally isolation of an androgenic 17-ketosteroid, adrenosterone, from adrenal tissue has been taken as evidence in favor of the secretion of such compounds by the normal gland.

Other workers, however, have believed that conversion of a C_{21} compound to an androgenic substance by peripheral tissues might account for the masculinizing effects seen after stimulation of the adrenal. The amounts of androgenic material found in adrenal tissue, even when the gland is largely made up of a masculinizing tumor, are very small. Since large amounts of 17-ketosteroids are excreted, it has been thought that such compounds as 17-hydroxycorticosterone and cortisone might be metabolized to these excretion products in other tissues of the body. The androgenic compounds isolated from adrenal tissue have likewise been considered to be possible intermediates in the production of the C_{21} steroids.

A comparison of the well known marked increase in urinary 17-ketosteroids following ACTH administration with the small conversion of exogenously administered C_{21} adrenal steroids to urinary 17-ketosteroids supports the hypothesis that the adrenal gland is able to secrete steroid compounds having the 17-ketone structure. This hypothesis is confirmed by the observation that 17-ketosteroids are present in the adrenal venous blood of the cow following stimulation of the adrenal cortex by ACTH. The same fraction has androgenic activity. It seems probable, therefore, that the androgenic effects and excretion of 17-ketosteroids which are seen after stimulation of the normal adrenal with ACTH are directly due to compounds secreted from the adrenal glands.

Summary. A fraction isolated from adrenal venous blood of cows has been demonstrated

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to have androgenic activity and to contain a compound giving the reaction for 17-ketosteroids. Its exact chemical structure has not been established due to the small quantity of the substance available for analysis.

Received July 18, 1951. P.S.E.B.M., 1951. v77.

Action of Calcium on Aerobic and Anaerobic Phosphorylation in Malignant and Certain Normal Tissues. (18939)

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In a previous communication(1) measurements of phosphorus uptake by homogenates of liver, brain, and tumor, and by the particulate and supernate fractions obtained by centrifuging such homogenates, were reported. The measurements were made under both aerobic and anaerobic conditions; the results indicated that phosphorylation could be effected by two different systems. In one system, associated chiefly with the particles and more prominent in liver than in tumor homogenates, phosphorus uptake was coupled with aerobic oxidation of succinate or other substrates of the tricarboxylic acid cycle; in the other system, associated chiefly with the supernate and quantitatively more important for the tumor homogenates, phosphorus uptake was coupled with anaerobic, glycolytic breakdown of hexose diphosphate.

Phosphorylation by the aerobic particulate system was, in confirmation of previous workers(2,3), blocked by 10^{-6} to 10^{-5} M 4,6-dinitro-o-cresol (DNC); in contrast, the phosphorylation by the glycolytic mechanism was not impaired by 10^{-4} to 10^{-3} M DNC. The present experiments deal with the sensitivity of these two phosphorylating systems to calcium ion.

Results. The effects of calcium upon phosphorus uptake by homogenates, particles, and supernate were measured with hexose diphosphate and pyruvate as substrate under exactly the same conditions as those described in the previous paper(1). Under these conditions part of the phosphorylation by the homogenate is associated with aerobic oxidation of pyruvate (chiefly by the particles) and part with glycolysis of hexose diphosphate (chiefly by supernate).

Calcium ion reduced the phosphorus uptake by particles from liver from 7.3 to –0.4 micromoles and by particles from brain from 8.5 to 1.5; phosphorus uptake by particles from the tumor, already low in the controls, was not further reduced by 0.0028 N calcium ion (Table I). The effect of calcium on phosphorus uptake by liver and brain particles under these conditions was a specific one, as oxygen consumption and lactate production were not significantly impaired at concentrations up to 0.0028 N Ca^{++}.

The phosphorus uptake by the supernate fraction from liver, brain, or tumor was not inhibited by calcium at concentrations up to 0.0028 N (Table I); there was also no impairment of oxygen consumption or lactate formation by these supernate fractions. The discrepancy between the phosphorus uptake of the tumor homogenate and the sum of the phosphorus uptakes of the washed tumor particles and supernate was found, as in the previous paper, to be due to the fact that when the tumor particles were shaken with a volume of buffered KCl equal to that of the original homogenate and recentrifuged, the wash supernate was found to contain an unexpectedly large amount of the glycolytic phosphorylating mechanism previously found in the original supernate. Since it appeared improbable that such large amounts of this

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