

from the urine of a man with a malignant tumor of the adrenal cortex. Engel⁴ isolated two crystalline compounds from the non-alcoholic ketonic fraction of normal male urine. Hirschmann⁵ obtained androstenone-17 from the urine of ovariectomized women. We have obtained similar crystallisates from various human urines.⁶

The following table gives the colorimetric titers of (1) the total neutral, (2) the ketonic neutral, (3) the alcoholic ketonic neutral and (4) the non-alcoholic ketonic neutral fractions of a set of pooled urines from non-cancerous and cancerous men and women. The urines were hydrolyzed by boiling for seven minutes after adding concentrated HCl to 15 per cent. by volume. The ketonic material was separated into alcohols and non-alcohols by half-esterification with succinic anhydride.

TABLE 1
THE COLORIMETRIC TITER OF URINARY KETOSTEROIDS IN
VARIOUS FRACTIONS OF POOLED HUMAN URINES.
ALL VALUES IN MGM EQUIVALENT OF
17-KETOSTEROID PER LITER

Source of urine	Total neutral fraction	Total ketonic fraction	Alcoholic ketonic fraction	Non-alcoholic ketonic fraction
Non-cancerous males (188 liters)	14.70	11.62	5.73	5.51
Cancerous males (476 liters)	3.90	3.68	1.65	1.73
Non-cancerous females . . . (146 liters)	5.69	5.38	2.66	2.00
Cancerous females . . . (231 liters)	3.99	3.58	1.74	1.52

It can be seen that roughly half of the titer of the ketones lies in the non-alcoholic fractions. It is interesting that these data on pooled urines confirm previous findings on individual specimens that cancerous persons of both sexes excrete approximately the same amounts of neutral ketosteroids, whereas in the non-cancerous persons there is a clear sex difference and a higher output than in cancerous persons.⁷

If the non-alcoholic ketosteroids are not excreted as conjugated compounds (it is difficult to see how they can be conjugated), then they should be found in full quantity in unhydrolyzed urine. Accordingly, we made a thorough ether extraction of freshly voided male urines (collected from 9 males and extracted within two hours of voiding). This produced 0.3 mgm 17-ketosteroid equivalent per liter of non-alcoholic ketone; the residue after acid hydrolysis yielded an

additional 3.2 mgm per liter and 5.1 mgm per liter of alcoholic ketosteroid. We satisfied ourselves by a number of recovery experiments that the separation by succinic anhydride can be successfully carried out on this micro-scale. As little as 50 micrograms of dehydro-androsterone may be isolated in 90 per cent. yield in the alcoholic fraction. The indications, therefore, are that the bulk of the non-alcoholic steroid material arises as a result of the hydrolysis employed.

Since the foregoing findings cast doubt on the probability that the non-alcoholic ketones are true excretion products we have conducted experiments on the effects of acid hydrolysis on androsterone and dehydroandrosterone. Preliminary data indicate that a considerable quantity of each is converted into non-alcoholic material which gives the Zimmermann color reaction. Butenandt and Dannenbaum⁸ obtained a chloroketone from urine which they consider a product of HCl hydrolysis. It is notable too that all the non-alcoholic urinary ketones thus far identified might conceivably arise by dehydration of the hydroxyketonic material in the course of acid hydrolysis.

The detailed data of this work and related investigation will be reported elsewhere.

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p-AMINO BENZOIC ACID, A VITAMIN

ABOUT a year ago, Woods and Fildes¹ reported the anti-sulfanilamide activity in vitro of p-aminobenzoic acid. In April, Woods² found that yeast extracts contain a substance which nullifies the inhibitory action of sulfanilamide on the growth of hemolytic streptococci, and presented circumstantial evidence that the yeast factor may be p-aminobenzoic acid. In December, Rubbo and Gillespie³ recovered p-aminobenzoic acid as the benzoyl derivative from yeast and concluded it to be a bacterial growth factor. Experiments conducted in this institute indicate that p-aminobenzoic acid, considered to be an essential metabolite for bacteria by Fildes,⁴ is a vitamin, namely, a chromotrichia factor for the rat and a growth-promoting factor for the chick.

One hundred black or piebald rats were placed at weaning age on the basal ration GH-1, consisting of Cerelese 70 per cent., casein 18 per cent., salts 4 per cent., agar 2 per cent., soybean oil 2 per cent., Crisco 2 per cent., and cod liver oil 2 per cent., and received

⁸ A. Butenandt and J. Dannenbaum, *Ztschr. Physiol. Chem.*, 229: 192, 1934.

¹ D. D. Woods and P. Fildes, 207th Meet. Biochem. Soc., U. of Sheffield, February 17, 1940; through *Chem. Ind.*, 59: 133, 1940.

² D. D. Woods, *Brit. Jour. Exp. Path.*, 21: 74, 1940.

³ S. D. Rubbo and J. M. Gillespie, *Nature*, 146: 838, 1940.

⁴ P. Fildes, *Lancet*, 238: 955, 1940.

³ H. Burrows, J. W. Cook, E. M. F. Roe and F. L. Warren, *Biochem. Jour.*, 31: 950, 1937.

⁴ L. L. Engel, *Am. Jour. Physiol.*, 129: P352, 1940.

⁵ H. Hirschmann, *Jour. Biol. Chem.*, 136: 483, 1940.

⁶ Unpublished data.

⁷ N. T. Werthessen and G. Pincus, *Am. Jour. Physiol.*, 129: P494, 1940.

daily $\frac{1}{2}$ ml of supplement S-8, a 20 per cent. ethanol solution containing per ml 80 γ each of thiamine hydrochloride, riboflavin and pyridoxine hydrochloride, 1 mg each of calcium pantothenate, nicotinic acid and inositol, and 6 mg of choline chloride. When definite graying of the fur had become apparent, 70 animals received a second daily supplement, namely 1 ml of preparation X-1, a 20 per cent. ethanol solution containing 3 mg/ml of p-aminobenzoic acid (E. K. #14, M. P. 182-4 with decomp.). A bluish discoloration of the skin, a typical first sign of growth of normally pigmented hair, was seen in from two to three weeks and black hair appeared within a month. The 30 control animals, not receiving supplement X-1, continued to show typical achromotrichia.

Chicks reared on the heated vitamin K-deficient ration, recently described,⁵ were found to show only a small gain (less than 100 gm) in weight and to die

within about a month, even when ample amounts of calcium pantothenate and of the vitamin K-active 2-methyl-1,4-naphthoquinone were fed. However, the addition of 300 γ of p-aminobenzoic acid per gm of ration resulted in better growth and longer survival times. In fact, 78 of 93 birds are still growing at the end of the second month and showed gains in weight of as much as 300 gm in spite of the severe dermatitis symptoms similar to the ones recently described by Hegsted *et al.*⁶

The experiments to date seem to permit the conclusion that p-aminobenzoic acid is one of the factors of the vitamin B complex. Detailed data will appear elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR MEASURING THE AREA OF SMALL IRREGULAR SURFACES OF THE HUMAN BODY

THE methods^{1, 2} previously described for measuring the area of parts of the human body are satisfactory for large or fairly regular surfaces. The area of a small irregular surface such as the pinna of the ear can not be accurately learned by these procedures. The method to be described proved satisfactory for such an area.

Brass plates of known areas, 4 and 10 sq. cm., were covered with a single layer of small lead discs of uniform diameter and thickness (1.02 mm diameter and 0.69 mm thickness) so placed as to reduce bare space to a minimum and held in place with petroleum jelly. The discs were removed, washed free from the jelly and weighed with an accuracy of 0.1 mg. The weight of lead discs necessary to cover one square centimeter could then be calculated. From five separate such measurements 0.601 gm of the discs was found to cover one square centimeter of flat surface. A brass model of known area and similar in shape to the postero-superior portion of the pinna was constructed. The surface of the model was covered with the lead discs and the weight of these discs measured. From the weight of these discs the area of the model was calculated with an error of no more than 2.8 per cent.

A negative cast of the postero-superior portion of

the pinna (the part studied is illustrated in Fig. 1) was made of a resilient moulage, Negocoll.³ Positive casts of dental stone⁴ were then made. The positive casts were covered with the lead discs. From the weights of the discs necessary to cover the casts the

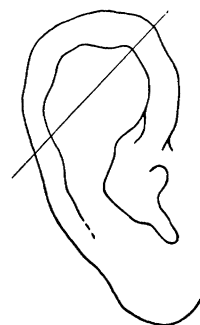


FIG. 1. Right pinna. Part of pinna studied is that part above the oblique line.

surface areas were calculated. Duplicate measurements were made in each case. The value of the dupli-

TABLE 1

AREAS OF THE POSTERO-SUPERIOR PORTION OF THE PINNA OF FIVE NORMAL WHITE ADULTS					
Subject No.	1	2	3	4	5
Sex	F	M	M	M	M
Age	50	27	30	32	40
Weight of the discs (gm)	13.60	13.28	13.21	13.02	13.86
Area (sq. cm)	8.168	7.981	7.944	7.826	8.319

⁵ S. Ansbacher, *Proc. Soc. Exp. Biol. and Med.*, 44: 248, 1940.

¹ E. F. Du Bois, "Basal Metabolism." 3rd ed. Lea and Febiger, Philadelphia, 1936.

² H. Isbell, "The Human Finger Tip: Surface Area and Volume Correlations." *Human Biol.*, 11: 536, 1939.

⁶ D. M. Hegsted, J. J. Oleson, R. C. Mills, C. A. Elvehjem and E. B. Hart, *Jour. Nutrition*, 20: 599, 1940.

³ A proprietary preparation of Kern Company, New York City.

⁴ Albastone—a preparation of S. S. White Dental Manufacturing Company.