

of healthy young guinea pigs. Such cystic conditions are, of course, frequently found in normal stock but here especially in old or unhealthy specimens.

The changed nutritive conditions in the reproductive organs of underfed animals cause circulatory congestion, and as was pointed out in a previous communication¹ such conditions suppress the oestrous changes and prevent ovulation in these animals. The congestion and the high pressure resulting therefrom seem to favor the proliferation of the epithelial lining of the epididymal tubules located near one pole of the ovary, and the accumulation of fluid within the lumen of the blind tubules.

The malnutrition expresses itself first within the ovary by a wholesale degeneration of developing follicles which seem to respond most delicately to changes in nutritive conditions. The congestion and follicular degeneration seem then to favor an overgrowth of the more resistant epididymal tubules which become distended and crowd out the parenchymatous portion of the ovary.

Uterine cysts seem to develop in the same way as those above as a response to the congestion resulting from malnutrition. The open mouths of the uterine glands make their cystic condition rare so that among hundreds of ovarian cysts of all sizes we have observed only one perfectly typical case of uterine cyst.

These experiments seem to indicate that ovarian and parovarian cysts represent growths of persistent embryonic tissue, and that an accompanying congestion and high pressure are necessary to the formation of typical cysts, and that these conditions may result from disturbed nutrition as is demonstrated by underfeeding the guinea pigs.

FROM THE PACIFIC COAST BRANCH.

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The synthesis of benzoyltaurin.

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It appears to be a specific function of several of the amino

¹ G. N. Papanicolaou and C. R. Stockard, *PROC. SOC. EXP. BIOL. AND MED.*, 1920, xvii, 143.

acids to combine with certain substances which the body is unable to oxidize. When benzoic acid and the numerous aromatic substances which are converted into benzoic acid in the body are ingested by man and certain other animals they are eliminated in the urine almost quantitatively as hippuric acid. Phenylacetic acid is voided in the urine of the dog and of the rabbit conjugated with glycocholic acid as phenaceturic acid¹ while in man it combines with glutamine and appears in the urine as phenylacetylglutamine.² The following also belong to the list of substances which undergo conjugation with glycocholic acid in passing through the body: furfural³ which in part is first oxidized to pyromucic acid and excreted as pyromucuric acid and in part combines with acetic acid and is eliminated as furfuralacrylic acid, α -methylthiophene⁴ which is oxidized to α -thiophenic acid and appears in the urine as thiophenuric acid, and α -methyl pyridin (α -picolin)⁵ which after oxidation to α -pyridin carboxylic acid passes into the urine as α -pyridinuric acid. The rôle which in animals is given to glycocholic acid is taken in birds by ornithin.⁶ Ingestion of benzoic acid by chickens leads to the appearance of ornithuric acid and similarly, pyromucic acid is conjugated with ornithin to give pyromucinornithuric acid. In certain instances the part of the conjugating amino acid is taken by cystein⁷ and by taurin. Halogen combinations of benzol and naphthalin are linked in the body with cystein and this substance, after acetylation of the amino group, appears in the urine combined with glucuronic acid. In the bile of most animals both glycocholic acid and taurin are found in combination with

¹ Salkowski, E., *Z. physiol. Chem.*, 1885, ix, 229.

² Thierfelder, H., and Sherwin, C. P., *Berichte*, 1914, xlvii, 2630. Thierfelder, H., and Sherwin, C. P., *Z. physiol. Chem.*, 1915, xciv, 1. Sherwin, C. P., Wolf, M., and Wolf, W., *J. Biol. Chem.*, 1919, xxxvii, 113. Shipley, G. J., and Sherwin, C. P., *J. Amer. Chem. Soc.*, 1922, xliv, 618.

³ Jaffé, M., and Cohn, R., *Berichte*, 1887, xx, 2311.

⁴ Jaffé, M., and Levy, H., *Berichte*, 1888, xxi, 3458.

⁵ Cohn, R., *Z. physiol. Chem.*, 1893, xviii, 112.

⁶ Jaffé, M., *Berichte*, 1877, x, 1925; 1878, xi, 406. Jaffé, M., and Cohn, R., *Berichte*, 1888, xxi, 3461. Totani, G., *Z. physiol. Chem.*, 1910, lxviii, 75. Suga, T., *Chem. Abstr.*, 1921, xv, 881.

⁷ Baumann, E., and Preusse, C., *Berichte*, 1879, xii, 806. *Z. physiol. Chem.*, 1881, v, 309. Baumann, E., and Schmitz, P., *Z. physiol. Chem.*, 1895, xx, 586. Jaffé, M., *Berichte*, 1897, xii, 1092.

cholic acid although in the bile of the dog and of the sheep taurocholic acid appears to be the only bile salt present.¹

No adequate theory has been advanced to explain the specific rôle which is thus played by these amino acids. It cannot be entirely a question of availability since probably all of the amino acids appear in the blood stream. The facts at hand are still too few to warrant an hypothesis. An attempt was made a number of years ago by Koelker and Amberg² to influence the normal course of the synthesis of hippuric acid. On simultaneous administration of benzoic acid and dl-leucin, benzoyl leucin did not appear in the urines of the experimental rabbits while the presence of hippuric acid and free benzoic acid was easily shown. The authors conclude that dl-leucin possesses a detoxifying effect on the action of benzoic acid although no explanation is offered as to the mechanism of this action.

ANIMAL EXPERIMENTS.

It appeared to us that since taurin behaves in nearly all respects like an amino carboxylic acid³ and that since both taurin and glycocoll are combined with cholic acid as bile salts, an opportunity was afforded for the possible substitution of taurin for glycocoll when benzoic acid is ingested. Taurin, when administered to the dog or to man, is not broken down but is excreted in the urine unchanged.⁴ An opportunity is thus afforded not only for the possible conjugation with benzoic acid in the body but also for the estimation of uncombined taurin in the urine. Benzoic acid and taurin were simultaneously fed to both man and to dogs. The dose of the latter substance was several times that required to combine with the amount of benzoic acid which was given. In the experiments of the first series estimations of total nitrogen, amino nitrogen, free and conjugated benzoic acid (Folin and Flanders),⁵ neutral sulfur, total sulfates and total sulfur were

¹ Schmidt, C. L. A., and Dart, A. E., *J. Biol. Chem.*, 1921, xlv, 415.

² Koelker, A. H., and Amberg, S., *J. Pharmacol.*, 1910-1911, ii, 59.

³ Schmidt, C. L. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1921, xviii, 204.

⁴ Schmidt, C. L. A., Von Adelung, E. and Watson, T., *J. Biol. Chem.*, 1918, xxxiii, 501. Schmidt, C. L. A. and Allen, E. G., *J. Biol. Chem.*, 1920, xlii, 55.

⁵ Folin, O., and Flanders, F. F., *J. Biol. Chem.*, 1912, xi, 257.

carried out. A portion of the urine was also used for the isolation of hippuric acid according to Dakin's method.¹ On account of the difficulty of removing pigments the method in our hands was not adapted for the quantitative estimation of hippuric acid but it did serve to establish the presence of this substance qualitatively. In the second series of experiments attempts were made to estimate benzoyltaurin directly. A volume of urine was evaporated to dryness and extracted with ethyl acetate in accordance with the procedure used by Dakin for the estimation of hippuric acid. After evaporating the ethyl acetate an estimation of neutral sulfur was carried out on the aqueous solution of the residue. The result of an experiment in which benzoyl taurin was added to normal urine indicated a recovery of 70 per cent.

The data indicate that no appreciable synthesis of benzoyltaurin takes place in the body when taurin and benzoic acid are simultaneously administered. The increase in neutral sulfur (62 per cent. of the taurin sulfur) and in amino nitrogen (60 per cent. of the taurin nitrogen) of Subject No. I agrees with the results of former work on the output of taurin when ingested by man. The experiments carried out on dog No. I also indicates a recovery ($S = 59$ per cent., $NH_2 = 66$ per cent.) or about 60 per cent. of the dose of taurin which was given. The figures for neutral sulfur in the experiments of series II are within the limits of the normal variability and indicate that benzoyltaurin was not present in amounts which can be estimated. The figures for benzoic acid indicate that this substance was almost quantitatively recovered. The presence of hippuric acid in the urine was established in all of the experiments.

SYNTHESIS OF BEZOYLTAURIN.

Baum² who prepared the benzoyl derivatives of a number of the amino acids by treating them with benzoyl chloride reports that he was unable to similarly synthesize benzoyltaurin. Later the substance was prepared by Gabriel and Heymann³ from

¹ Dakin, H. D., and Hawk, P. B., "Practical Physiological Chemistry," Philadelphia, 1918, p. 543.

² Baum, J., *Z. physiol. Chem.*, 1885, ix, 465.

³ Gabriel, S., and Heymann, P., *Berichte*, 1890, xxiii, 157. Gabriel, S. and Colman, J., *Berichte*, 1911, xlv, 3628.

μ -phenylthiazolin. In the synthesis of benzoyltaurin use was made of a buffer substance such as NaHCO_3 or NaOOCCH_3 instead of NaOH which was employed by Baum to neutralize the HCl which is set free during the course of the reaction. The procedure follows essentially the method used by Fischer¹ for the benzylation of other amino acids.

Five grams of taurin in solution were shaken with 10 grams of benzoyl chloride in the presence of sufficient NaHCO_3 to keep the reaction slightly alkaline. The benzylation was continued until no nitrogen was obtained when a test portion was treated with HNO_2 . The solution was made just acid by addition of HCl , cooled in the ice chest and the benzoic acid was removed by filtration. The filtrate was evaporated to dryness at a low temperature and the residue was extracted with petroleum ether to remove traces of benzoic acid. The residue was then extracted with dry ethyl acetate in which solvent benzoyltaurin is slightly soluble. Unlike hippuric acid and other benzyolated amino acids benzoyltaurin is not easily crystallizable either in the free state or as the sodium salt, hence purification is difficult. It is very soluble in water and only slightly soluble in alcohol. Analysis of our product gave the following values:

	Found.	Calculated for $\text{C}_6\text{H}_5\text{COHNCH}_2\text{CH}_2\text{SO}_3\text{Na}$.
Nitrogen.....	5.5%	5.6%
Sulfur.....	13.4%	12.8%
Sodium (as Na_2SO_4).....	9.8%	9.2%
α - NH_2	0.3%	—

TABLE I.

THE SIMULTANEOUS ADMINISTRATION OF SODIUM BENZOATE AND TAURIN.

Dose.	α - NH_2 , Mg.	Total Sul- fur, Gm.	Total Sul- fates, Gm.	Neu- tral Sul- fur, Gm.	Total Nitro- gen, Gm.	Free Ben- zoic Acid, Gm.	Conju- gated Ben- zoic Acid, Gm.
<i>Dog. No. 1.</i>							
.....	68	0.152	0.082	0.070	2.8		
6 gm. taurin, 6.8 gm. benzoic acid.....	330	1.155	0.092	1.063	3.5	0.58	5.82
.....	187	0.260	0.016	0.144	3.2		
.....	71	0.144	0.041	0.103	3.2		

¹ Fischer, E., *Berichte*, 1899, xxxii, 2451.

<i>Human Subject</i>							
.....	141	0.938	0.770	0.168	13.6	0.11	0.44
8 gm. taurin, 3.76 gm. benzoic acid.....	692	2.360	0.888	1.472	14.4	0.10	3.56
.....	163	1.220	1.002	0.218	15.3	0.09	0.38

TABLE II.

THE SIMULTANEOUS ADMINISTRATION OF SODIUM BENZOATE AND TAURIN.

Dose.	Free Benzoic Acid, Gm.	Conjugated Benzoic Acid, Gm.	"Neutral Sulfur," Gm.
<i>Dog. No. 2.</i>			
.....	0	0.15	0.009
3.76 gm., benzoic acid.....	0.19	3.36	0.032
3.76 gm. benzoic acid, 11.25 gm. taurin ..	0.77	3.43	0.013
9.04 gm. benzoic acid, 27.0 gm. taurin ..	2.50	2.44	0.010
.....	1.61	2.32	0.007
<i>Human Subject.</i>			
.....	0.04	0.37	0.014
1.51 gm. benzoic acid.....	0.05	1.50	0.014
1.51 gm. benzoic acid, 4.5 gm. taurin ...	0.07	1.55	0.014

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Blood-sugar studies.

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In a recently reported study of certain blood-sugar phenomena McLean and de Wesselow¹ have attempted to explain the nature of the blood-sugar curve obtained after the ingestion of glucose by man. As a result of the comparison of the curves of a normal and of a diabetic individual they suggest that the existence of an alimentary hyperglycemia in the normal awakens and stimulates the glycogen-forming mechanism to such activity that not only is the rising hyperglycemia checked but that the blood-sugar concentration is rapidly brought down to normal or below, thus accounting for the rapid rise and fall of blood sugar which is the characteristic response of the normal to the ingestion of glucose.

¹ McLean, H. and de Wesselow, O. L. V., *Quart. Jour. Med.*, 1921, xiv, 103.