	p_H* 6.9.	p_H* 7.5.	p_H† 7.9.	p_H† 8.4.	p_H† 8.9.	p_H† 9.1.	p_H† 9.4.
Sample 1	78%	85%	79%	68%	66%	59%	52%
Sample 2	121%	124%	123%	113%	103%	79%	76%
Sample 3	362%	363%	350%	335%	332%	307%	290%
Sample 4	350%	353%	334%	326%	328%	299%	271%

* Casein solution p_H value determined using brom-thymol blue color disc in comparator.

† Casein solution p_H value determined using thymol blue color disc (alkaline range) in comparator.

The results obtained indicate clearly that the hydrogen-ion concentration of the casein solution must be taken into consideration in carrying out this method in order to insure consistent results. Data obtained indicate that a preference is shown for casein solutions with a p_H value of 7.5. However, pancreatin samples assayed using casein solutions with p_H values of 6.9 and 7.9 show little variance from the results obtained with the former casein solution mentioned. We would, therefore, suggest that the casein solution for use in this method be adjusted to a p_H value not less than 7.0 and not more than 8.0.

The advantages of this method over the present U. S. P. method are:

(*a*) A definite end-point is obtained.

(*b*) It is much shorter, providing a supply of the preserved casein solution is kept on hand.

(*c*) More consistent results can be obtained, especially with the higher strength samples of pancreatin.

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ACCELERATED PRODUCTION OF SPECIFIC URINARY PIGMENTS BY DRUG ADMINISTRATION.*

I. EFFECT OF PHENYLDIMETHYLPYRAZOLON ON UROBILIN FORMATION.

BY FREDERICK G. GERMUTH.

INTRODUCTION.

The relation of chemical constitution to physiological activity in synthetic therapeutic agents has long been a subject of fascinating interest. Aside from purely theoretical aspects, the action of chemical agents upon the animal organism, and upon mankind in particular, is avowedly of the greatest importance. A knowledge of the relationship obtaining is of fundamental significance and importance—not alone to those contemplating the exploration of this vast field, but is certainly of no less import to the practitioner whose duty it is to prescribe the employment of this particular class of medicinal substances. It is believed, therefore, that the paper here presented—the first of a contemplated series dealing with urinary pigment formation engendered by the administration of specific organic medicinals—will be of interest alike to the members of the medical and pharmaceutical professions as well as to the chemist and others engaged in scientific work.

In a previous research,[1] the author has furnished data describing the pro-

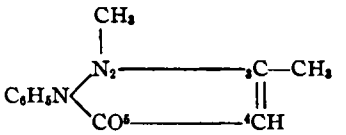
* Contribution from the Division of Research, Bureau of Standards, City of Baltimore.

[1] Germuth, *American J. Pharm.*, 99 (1927), No. 11.

nounced action of the sulphone, diethylsulphonemethylethylmethane (Trional) upon the production of the pigment, hæmatoporphyrin. This was followed by a paper dealing with the accelerating effect of the most therapeutically active member of this series of alkylated soporifics, diethylsulphonediethylemethane (Tetronal), upon the porphyrin, in which attention was accorded the fact that an increase in ethyl groups in the molecular structure of this aliphatic compound imparts, in addition to a high degree of therapeutic activity, properties which result in the formation in the system of a proportionately greater amount of hæmatoporphyrin than that observed when either sulphonal or trional is ingested.[1] This work prompted the desire to instigate further investigations of like character in the effort to ascertain the ultimate effect of certain compounds upon the production of physiological bodies of known, definite srructure.

HISTORICAL.

It will be recalled that the constitution which Knorr[2] first ascribed to a tetrahydroquinoline derivative was the cause for testing the so-called dimethyloxyquinizine, the antipyretic effect of which was found to be most pronounced.[3] At a later period this compound was recognized as phenyldimethylpyrazolon,[4] better known at present, perhaps, as antipyrine—possessing the structural formula:



While Michaelis[5] affirms that another formula may also be ascribed to this compound, that given is considered as more correctly embodying the chemical constitution of phenyldimethylpyrazolon.

It may be of interest to observe at this place that, while antipyrine was once widely prescribed in conjunction with sodium bromide in the treatment of disorders of nervous origin (its anæsthetic action on nerve endings rendering its application rather desirable in these conditions), its employment in this respect has been considerably curtailed by the advent of derivatives, such as 4-dimethylaminoantipyrine (Pyramidon), Pyrosal and certain others in which alkylation of the nitrogen has taken place.

METHOD.

The method of Hoppe-Seyler[6] was applied in the estimation of urobilin in the samples of normal and pathological urines under examination, insuring, it is believed, a high degree of accuracy. The essential procedure is as follows:

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[1] Germuth, *Indian Med. Gaz.*, 64 (1929), No. 9.
[2] Knorr, *Ber.*, 17 (1884), 546, 2032.
[3] Filehne, *Z. klin. Med.*, 7 (1884), 6.
[4] Knorr, *Ann. Chim.*, 238 (1887), 137.
[5] Michaelis, *Ibid.*, 320 (1902), 1.
[6] Hoppe-Seyler, *Virchow's Arch.*, 124.

One hundred ml. of urine are acidified with 1:4 H_2SO_4, and then saturated with $(NH_4)_2SO_4$. After one hour has elapsed, the precipitate is collected on a filter, washed with a saturated solution of $(NH_4)_2SO_4$, and repeatedly extracted with a solution consisting of equal portions of C_2H_5OH and $CHCl_3$, after pressing firmly. The filtered solution is treated with H_2O in a separatory funnel until the $CHCl_3$ separates well and becomes clear. The $CHCl_3$ solution is evaporated on the water-bath in a weighed beaker, the residue dried at 100° C., and then extracted with $(C_2H_5)_2O$. The ethereal extract is filtered, the residue on the filter dissolved in C_2H_5OH, and transferred to the beaker and evaporated; then carefully dried and weighed.

EXPERIMENTAL.

Recognition was accorded the fact that the urinary pigment here considered is present in increased quantities in disorders accompanied by fever; in cardiac diseases, atrophic cirrhosis of the liver, and certain other physiological conditions. Adequate precaution was taken to obviate any discrepancies that might accrue as a result of the presence of factors that might tend to introduce errors in the experimental portion of this work.

Ten samples of urine, representing the 24-hour output of patients for whom the antipyretic had been prescribed, and to whom it had been administered in dosages of 15 grains per day, intermittently, for a period of ten days, were collected on the eleventh day. Ten additional samples, taken from patients whose hospitalization had not required the use of antipyrine, and who were, apparently, free of any disorder that might cause an increase in the production of urobilin, were collected and subjected to the same treatment given the physiological urines.

Table I furnishes the data obtained by careful analysis of the samples of normal body fluid.

TABLE I.—DETERMINATION OF UROBILIN IN NORMAL URINE.

Amount expressed in mg. per liter.

Sample No.	1	2	3	4	5	6	7	8	9	10
Quantity	45	34	38	29	45	33	78	42	37	49

Average—43 mg.

The ten samples of urine obtained from patients who had been given the antipyrine treatment were next submitted to chemical examination, and the results given in Table II observed.

TABLE II.—DETERMINATION OF UROBILIN IN PATHOLOGICAL URINE.

Amount expressed in mg. per liter.

Sample No.	1	2	3	4	5	6	7	8	9	10
Quantity	98	110	108	95	138	106	142*	89	104	126

Average—112 mg.

* Patient developed a decided icterus.

REMARKS.

Consideration of the results tabulated justify the belief that the continued employment of phenyldimethylpyrazolon over a period of ten days or less, promotes the disintegration of erythrocytes, causing a condition characterized by the appearance of excessive amounts of urobilin in the urine. While certain forms of disease also tend to emphasize this condition, the action of the synthetic appears to be most readily discerned. As will be shown in a later paper, acetanilid exerts a similar effect, but fails to act as quickly as the compound here considered.

While some degree of variation has been experienced in the different results, it is obvious that the samples of urine obtained from the physiological sources contain a marked excess of urobilin over that generally encountered in so-called normal urines.

SUMMARY.

(*1*) The administration of phenyldimethylpyrazolon over a certain period of time occasions the appearance of an abnormal quantity of urobilin in the system.

(*2*) It is believed that the disintegration of erythrocytes is considerably augmented by continued ingestion of the antipyretic, resulting in an increased proportion of the urinary pigment.

(*3*) Data are furnished, showing the relative amounts of the body existing in normal and physiological samples of urine.

(*4*) Future work on the effect of certain of other therapeutic agents upon urobilin production, is contemplated.

ACKNOWLEDGMENTS.

The author desires to acknowledge, with thanks, the coöperation extended him by Harry Goldsmith, M.D., physician-in-chief, Psycopathic Department of Baltimore City Hospitals, from whom a number of the samples of urine utilized in the tests were obtained, and the assistance rendered by Nelson T. Carey, M.D., of the staff of Mercy Hospital,[1] in furnishing samples of urine which were previously examined and employed for control purposes.

COMMENTS ON SOME TESTS AND ASSAYS OF THE U. S. P. X.*

BY HERBERT C. KASSNER.

For the past two years, part of my work has been to conduct a course of laboratory instruction for large classes of third year students, candidates for the degree of Pharmaceutical Chemist; part of the course consists in the carrying out of many U. S. P. tests and processes. It has been repeatedly made evident to me that certain tests and assays caused considerable difficulty and seemed in need of improvement; for example, on introducing impurities into certain substances, even in reasonably large proportions, it appeared impossible to detect them by the prescribed U. S. P. tests; again, some tests appeared to be unworkable unless modifications were made. It seemed advisable to call attention to the difficulties encountered and for this reason this paper has been written.

The list of subjects criticized does not pretend to be by any means comprehensive; it contains only those tests and assays which were included as a part of the course because of their importance from the educational point of view and which were the cause of trouble in one way or another. If a systematic search were made of the Pharmacopœia, doubtless many more difficulties on similar lines would be encountered.

All the tests commented upon have been carefully examined, many series of experiments being carried out and suggestions for modifications made wherever possible. In some cases it is merely the wording of the Pharmacopœia which has been

[1] At present on the Staff of Johns Hopkins Hospital.

* Scientific Section, A. PH. A., Rapid City meeting, 1930.