come the depressant effects of the drug.

The wide range in sexual potency of our subjects was in part the result of antecedent conditions to which they were subjected in an experiment by one of us (P. G. Z.) (*5*). After weaning, 11 of the rats had been reared under unfavorable conditions (that is, they had been given electric shock for approaching a receptive female and had been subsequently reared in isolation, or they had been reared entirely in isolation). Six rats had been reared under more favorable conditions (that is, they had been given no electric shock when they were with a receptive female and had been reared in cohabitation with other males or with females). In order to increase the number of subjects in testing the differential effects of previous sexual experience, eight pretest rats were added to this comparative study. These animals had been reared under the same conditions as the others (five under favorable conditions, three under unfavorable ones) but were given four *ten*-minute tests and were not given a no-drug test between the two drug tests.

In the various measures of sexual performance, the depressant effects of chlorpromazine were found to be approximately equal among animals reared under these two sets of conditions. On the other hand, the stimulating effects of caffeine were evident only among animals reared under the more favorable conditions. These animals copulated almost twice as many times when they were under caffeine as when they were under no drug and showed an increment, under caffeine, in number of mounts, in copulatory rate, and in percentage that copulated and ejaculated. Among the animals reared under unfavorable conditions, caffeine produced only a slight increment in copulatory rate and a slight decrement in all of these other measures. The effect of rearing conditions on the caffeine-versus-no-drug difference was statistically significant for number of copulations ($p < 0.02$) and almost significant for number of mounts ($p < 0.10$), according to the Mann-Whitney rank test. The different effects of caffeine might be explained by the hypothesis that the stimulating effects of caffeine enhance whatever behavior tendency is dominant in the situation. If the conditions of rearing have been favorable, positive sexual responses will generally be dominant; if the conditions of rearing have been unfavorable, inhibitory or incompatible responses may be dominant.

The study described in the present report confirms and extends the finding by Soulairac and Coppin-Monthillaud of the stimulating effect of caffeine on sexual behavior—even of a smaller dose. A depressant effect of chlorpromazine was also found. The use of non-drug tests and of animals with variation in previous sexual experience permitted further observations on effects of the drugs.

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23 September 1957

Electromigration on Filter Paper of Uric Acid from Serum and Synovial Fluid

It has long been a matter of discussion whether uric acid is present in human serum as a free molecule or is bound to other components such as proteins. Ultrafiltration and dialysis have given controversial results in this connection (*1*). Zone electrophoresis on filter paper offers a new approach to the study of the problem.

Previous reports from our laboratory (*2*) have shown that several techniques can be followed for identification and evaluation of uric acid on the strip of filter paper. The most practical technique proved to be direct treatment of the strip with silver nitrate and sodium lactate; uric acid appears, then, as a brown band on a white background. While this method is suitable for detecting the position of the spot of uric acid, quantitative data are more readily obtained by elution and chemical (*3*) or ultraviolet absorption analysis.

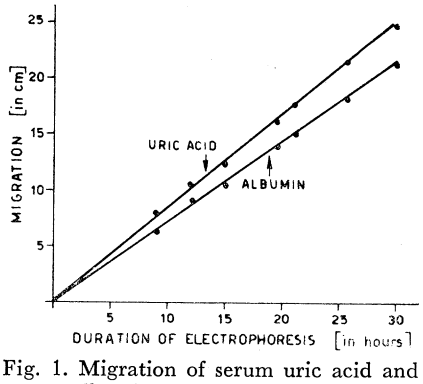

Fig. 1. Migration of serum uric acid and serum albumin as a function of duration of electrophoresis, in standard conditions of potential gradient (2.5 v/cm) and temperature (15°C), in Veronal at pH = 8.6, $\Gamma/2 = 0.05$. Each point represents an average of four experiments. In calculating distance of migration, it has been assumed that the starting point corresponds to the band of gamma globulin.

Paper electrophoresis was performed in accordance with the method of Durrum, as modified by Flynn and De Mayo (*4*). Different buffer solutions were tried: Veronal at pH = 8.6, $\Gamma/2 = 0.10$, 0.05, and 0.025, respectively; phosphate at pH = 7.2, $\Gamma/2 = 0.05$; acetate at pH = 5.4, $\Gamma/2 = 0.05$; glycine-NaOH at pH = 9.7, $\Gamma/2 = 0.05$. The best results, as far as resolution of protein components and uric acid is concerned, were obtained with Veronal buffer at pH = 8.6, $\Gamma/2 = 0.05$. Whatman No. 31 extra-thick filter paper was used.

Forty-two sera from normal subjects and from hyperuricemic patients with gout, nephritis, and leukemia and ten samples of synovial fluid from gouty and rheumatic subjects were studied. Control experiments were run with pure uric acid solutions of different concentration (from 3 to 20 mg percent). The results obtained can be summarized as follows:

1) Uric acid present in serum and synovial fluid showed the same electrophoretic behavior as uric acid in free solution, in all the conditions of pH, ionic strength, potential gradient, temperature, and duration of migration that were tried. A single band was always obtained for this substance. Though we cannot rule out entirely the possibility that there was a dissociation of labile bonds between uric acid and other serum components, it is not likely that such a dissociation would have occurred under the mild conditions of our experiments.

2) The mobility of uric acid, as judged from the electrophoretic migration on filter paper, proves to be slightly higher than that of serum albumin, being about 15 percent greater (Fig. 1).

3) No change of the electrophoretic behavior of uric acid was observed after general or intra-articular administration of drugs that are active in modifying uricemia (prednisone, phenylbutazone, probenecid, pyrazinamide).

Our results seem to indicate that uric acid is not bound to other serum components and is not present in a particular chemical form; several drugs that act on uric acid metabolism and excretion fail to modify the physical or chemical properties of this substance in the blood.

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4 November 1957

Development of Phenylalanine Hydroxylase in Mammalian Liver

Recent studies of the mechanism of conversion of phenylalanine to tyrosine in the soluble fraction of liver homogenates have established that the reaction requires the participation of two discrete enzymes in addition to reduced pyridine nucleotide (*1, 2*) and an as yet unidentified cofactor (*3, 4*). One of the enzymes can be prepared from tissues that do not hydroxylate phenylalanine (for example, kidney, brain, spleen); this suggests that a reaction of more general nature is involved. The second enzyme, which is specific, has been found only in liver. Wallace *et al.* (*5*) and Mitoma *et al.* (*6*) have recently demonstrated that the livers of phenylketonuric individuals lack this specific enzyme, thus strengthening the prevailing concept regarding phenylketonuria—namely, that the syndrome is associated with a specific congenital inability to oxidize phenylalanine to tyrosine.

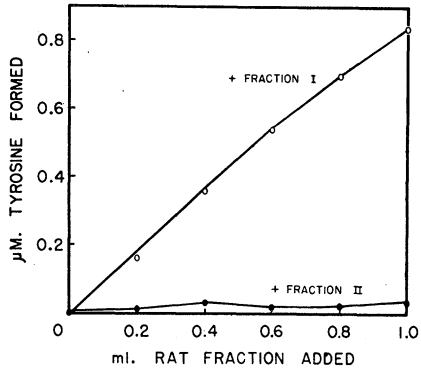

Fig. 1. Effect of rat liver fractions on phenylalanine hydroxylation in the liver of the fetal pig. Beakers containing 1.0 ml of the soluble fraction of fetal pig liver (17.2 mg of protein), 150 μmole of potassium phosphate buffer (*p*H 6.8), 2 μmole of DPNH, 2 μmole of L-phenylalanine, rat liver fractions as indicated, and 0.15*M* KCl to make a total volume of 3.0 ml were shaken for 60 minutes at 35°C. Tyrosine formed was measured colorimetrically. Rat fraction I, 3.3 mg of protein per milliliter; rat fraction II, 7.3 mg of protein per milliliter; these fractions individually formed only traces (less than 0.01 μmole) of tyrosine in 60 minutes.

Investigations on the development of this enzyme system in fetal, newborn, and adult rats have established the fact that the over-all reaction is virtually absent in fetal liver and in the livers of rats less than 24 hours old. In these experiments, 1.0 ml soluble fractions of liver (prepared from 33 percent homogenates in 0.15*M* KCl by centrifugation at 105,000*g* for 30 minutes) were shaken at 35°C for 60 minutes with 150 μmole of potassium phosphate buffer (*p*H 6.8), 2 μmole of L-phenylalanine, 2 μmole of reduced diphosphopyridine nucleotide (DPNH), and 5 μmole of nicotinamide in a total volume of 1.75 ml; the tyrosine formed was measured colorimetrically (*7*).

Under these conditions, preparations from adult animals and from animals 4 to 12 days of age converted all or most of the added phenylalanine to tyrosine, while preparations from fetal or newborn animals were essentially inactive. These inactive preparations could not be activated by substitution of TPNH (reduced triphosphopyridine nucleotide) for DPNH, or by increasing the amount of reduced pyridine nucleotide either by direct addition or by supplementing the reaction mixture with appropriate reducing systems (glucose-6-phosphate and yeast glucose-6-phosphate dehydrogenase with TPN; lactate and muscle lactic dehydrogenase with DPN). The ability to hydroxylate phenylalanine was also absent or negligible in livers of fetal pigs and fetal rabbits.

Experiments were carried out in order to determine which of the components is absent or inactive in fetal liver. Fractions I and II were prepared from adult rat livers by the procedure of Mitoma (*2*); each of these preparations alone was essentially inactive, while the two combined, when fresh, gave high rates of phenylalanine hydroxylation. Addition of rat fraction I, containing the specific enzymic component, to the inactive soluble fraction of fetal pig liver yielded a highly active combination, the level of activity being directly related to the amount of fraction I added (Fig. 1). Addition of fraction II from rat liver had essentially no effect on the fetal liver system. Similarly, supplementing the soluble fractions from livers of fetal rats or rabbits with fraction I resulted in high activity, while addition of fraction II was ineffective.

The absence of phenylalanine hydroxylation in fetal liver thus results from the same deficiency as that observed in the livers of patients with phenylpyruvic oligophrenia—that is, the specific enzymic component of the system, present in rat fraction I, is apparently not present in fetal liver.

Further, although fraction I has been described as being quite labile (*2*), it was found that aged or dialyzed preparations of this fraction, which were in fact inactive when assayed with fraction II, were still active in supplementing the fetal liver system. However, if the fetal liver preparation was well dialyzed, addition of aged or dialyzed fraction I was only slightly effective. These results indicate that fraction I contains two components, one of which is rapidly lost on aging or dialysis, and that this labile component is present in fetal liver. Loss of this component on dialysis, as well as on aging, suggests a factor of low molecular weight, which may be the auto-oxidizable cofactor described by Kaufman (*4*).

These findings lend support to the conclusion that the component missing in fetal liver, which is supplied by rat fraction I, is the specific hydroxylation enzyme. It is of interest to note that some other enzymes whose deficiency in postnatal life is associated with disorders of incomplete metabolism are also not active in fetal tissues; those investigated include the enzymes of tyrosine oxidation (*8*) and glucose-6-phosphatase (*9*). This relationship is being studied further in this laboratory (*10, 11*).

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10. Experiments similar to those described above were carried out with the soluble fractions of livers from two premature infants (liver samples obtained at autopsy, 9 and 2 hours after death). As with the fetal tissue, these preparations were inactive alone, active when rat fraction I was added, and not affected by rat fraction II. Although these results suggest that the liver of the premature infant also lacks the specific hydroxylation enzyme, the possibility exists that the time which elapsed between death and the enzyme assays was sufficient for specific autolysis of this enzyme.

11. These studies were supported in part by grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health (A-389 C3R); the Association for the Aid of Crippled Children; and the Damon Runyon Memorial Fund.

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