hours before and 1 and 10 hours after methanol injection. In a second study employing four control and four experimental rats, the methanol dosage was raised from 2 to 3 g/kg. In none of these experiments was there sufficient difference between the methanol disappearance from the bloods of treated and nontreated rats to indicate that AT significantly inhibits methanol metabolism *in vivo*.

In view of the disagreement between results obtained *in vitro* and *in vivo*, one is obliged to consider the following possibilities: (i) AT is not an inhibitor of liver catalase *in vivo*; (ii) liver catalase is much more effective in its role as a catalyst of methanol oxidation *in situ* than it is *in vitro*, in which case the small amount of uninhibited catalase remaining after AT treatment is still sufficient to mediate adequate methanol metabolism; and (iii) catalase does not participate in the *in vivo* metabolism of methanol in the rat.

G. J. MANNERING
R. E. PARKS, JR.*
Department of Pharmacology and Toxicology, University of Wisconsin Medical School, Madison

References and Notes

1. For detailed discussions of both sides of the question the reader is referred to the comments of the following authors: R. Lemberg and J. W. Legge, *Hematin Compounds and Bile Pigments* (Interscience, New York, 1949), pp. 415–419; B. Chance, *Advances in Enzymology* (Interscience, New York, 1951), pp. 185–188; G. R. Bartlett, *Quart. J. Studies Alc.* 13, 583 (1952); E. Jacobsen, *Pharmacol. Revs.* 4, 107 (1952); O. Röe, *ibid.* 7, 399 (1955).
2. R. K. Bonnichsen, *Acta Chem. Scand.* 4, 715 (1950).
3. D. Keilin and E. F. Hartree, *Proc. Roy. Soc. (London)* B119, 141 (1936); *Biochem. J.* 39, 293 (1945).
4. W. G. Heim, D. Appleman, H. T. Pyfrom, *Science* 122, 693 (1955).
5. The 3-amino-1,2,4-triazole was generously supplied by the American Cyanamid Co., Agricultural Chemical Division. It was purified twice according to the directions given by that company, specifically, by placing the compound in the thimble of a Soxhlet extractor and extracting with a mixture of methanol and benzene in the proportions of their azeotrope (39.6 percent by weight methanol and 60.4 percent by weight benzene). The product crystallizes in the boiler of the Soxhlet apparatus.
6. R. N. Feinstein, *J. Biol. Chem.* 180, 1197 (1949).
7. P. A. J. Strittmatter, "The biological oxidation of methanol and formaldehyde, thesis submitted to Harvard University, 1 Dec. 1953.
8. D. A. MacFadyen, *J. Biol. Chem.* 158, 107 (1945).
9. The crystalline beef liver catalase was purchased from Worthington Biochemical Corp., Freehold, N.J. A 1/5 solution of this suspension was made using isotonic KCl containing a trace amount of NH_4OH.
10. One K unit represents the monomolecular velocity constant extrapolated to zero time as described by J. B. Sumner and A. L. Dounce [*Methods in Enzymology* (Academic Press, New York, 1955) vol. 2, p. 779].
11. The glucose oxidase was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio.
12. K. Agner and K. E. Belfrage, *Acta Physiol. Scand.* 13, 87 (1947).

* Scholar in medical science of the John and Mary Markle Foundation.

12 August 1957

Response of Weaver Finch to Chorionic Gonadotrophin and Hypophysial Luteinizing Hormone

The specificity of the weaver-finch (*1*) feather reaction for luteinizing hormone has been described by Witschi (*2*), and the work with this assay has been reviewed by him recently (*3*). Preparations of luteinizing hormone (LH) from beef or sheep hypophyses give positive responses at dose levels far below the minimal effective doses of conventional rat assays. The weaver-finch unit for a standard LH preparation (Armour lot No. 227-80), for example, is 5 μg. In rat assays based on augmentation of follicle-stimulating hormone (FSH), the dose required to elicit a positive response with the same material is 1 mg (*4*).

In addition to its high degree of sensitivity, the weaver-finch test possesses the important advantage of being unaffected by the presence of nonluteinizing gonadotrophins. Doses of prolactin (LTH) as high as 5.0 mg fail to give a positive reaction. When this quantity of prolactin is added to the LH standard, the activity of the combined preparation proves to be identical with that of the LH component (Table 1). This same principle applies when combinations of hypophysial FSH and LH are prepared and tested in comparison with the LH factor alone. The FSH used in the experiment reported in Table 1 is inactive at a dose of 0.25 mg. Adding this amount of FSH to graded doses of LH has no measurable effect on the threshold for pigment deposition in the feathers. When the FSH preparation itself is assayed, a dose of 1.0 mg proves to have positive weaver-finch activity. This would seem to result from a small degree of LH contamination in the FSH sample. At this high total dose of FSH (1.0 mg) a 0.5 percent contamination with LH (0.005 mg) would account for the positive reaction. On this basis, 0.75 mg of FSH (inactive, when given alone) would contain LH contamination equivalent to the activity of about 0.003 mg of the LH standard. This factor, together with one-half the minimal effective dose of the LH standard (0.0025 mg), should exceed the threshold for weaver-finch activity. When these two subeffective doses of FSH and LH, respectively, are given simultaneously, this result is obtained (Table 1). The latter finding does not violate the principle that LH activity in the weaver-finch test is not affected by extraneous FSH present. In the combination employed, the FSH preparation simply acts as a carrier for the small amount of LH necessary to bring the total active material up to the required minimal effective dose.

The fact that gonadotrophins lacking LH activity do not synergize or augment in the weaver-finch test for LH is of particular importance in the testing of clinical material as well as in procedures designed to ascertain the purity of hypophysial extracts. When the hypophysectomized male rat is used in assays for LH, it is conceivable that the presence of prolactin in the sample would influence the size of the ventral prostate. Studies reported by several different laboratories indicate that LTH can enhance the responses of the ventral prostate and other sex accessories to LH in the hypophysectomized male rat (*5*). In the case of the immature or hypophysectomized female rat, the ovarian response to FSH in the test material is considerable and probably influences the interpretation of specific LH effects. It has never been established whether or

Table 1. Weaver-finch assay of hypophysial extracts and human chorionic gonadotrophin.

Material and dose	Weaver-finch reaction
Hypophysial extracts	
LH* (0.0025 mg)	−
LH* (0.005 mg)	+
LTH† (5.0 mg)	−
LH* (< 0.005 mg) and LTH† (5.0 mg)	−
LH* (0.005 mg) and LTH† (5.0 mg)	+
FSH‡ (0.25 mg)	−
FSH‡ (0.75 mg)	−
FSH‡ (1.0 mg)	+
LH* (< 0.005 mg) and FSH‡ (0.25 mg)	−
LH* (0.005 mg) and FSH‡ (0.25 mg)	+
LH* (0.0025 mg) and FSH‡ (0.75 mg)	+
Chorionic gonadotrophin (CGH)	
Pregnancy urine, 2nd mo of gestation (0.25 ml)	+
Pregnancy serum, 2nd mo of gestation (0.25 ml)	+
Normal male urine ultrafiltrate (6-hr concentrate)	−
Normal male urine ultrafiltrate (> 6-hr concentrate)	toxic
Male urine from patient with seminoma (0.25 ml)	+
Above male, after orchiectomy (2-hr concentrate)	−
CGH, Ayerst No. 29075 (30 IU)	−
CGH, Ayerst No. 29075 (60 IU)	+
CGH, Scheifflin No. 990331 (30 IU)	−
CGH, Scheifflin No. 990331 (60 IU)	+
CGH, Roche Organon No. 412018 (30 IU)	+
CGH, Roche Organon No. 412018 (60 IU)	+

* Luteinizing hormone, Armour lot No. 227-80. † Prolactin, Squibb lot No. 4B74978. ‡ Follicle-stimulating-hormone, Armour lot No. 377-200.

not the response of the theca interna to LH is independent of the state of development of the entire follicular apparatus. Assays dependent on increases in ovarian weight in female rats can be considered only as measures of total gonadotrophins.

Nonhypophysial luteinizing hormones also react positively in the weaver-finch test. This was first demonstrated with the use of pregnant mare's serum (*3*) and could be shown also with human chorionic gonadotrophin (*6*). Small quantities (0.25 ml) of pregnancy urine or blood serum from women in the second month of gestation cause positive feather reactions in the weaver finch (Table 1). The minimal effective dose has not yet been established for normal pregnancy urine or serum from women at various stages of gestation. The collection of these data in normal pregnancies may prove to be of value for the simple, rapid analysis of urines or blood sera from women with suspected deviations from normal chorionic gonadotrophin titers during pregnancy. Normal male urine is negative for LH by the weaver-finch test, but urine extracts from men with hormone-producing seminomas give positive responses. For the case reported in Table 1, several urine samples collected prior to orchiectomy were consistently positive by the weaver-finch assay, containing levels of luteinizing hormone (presumably of the chorionic gonadotrophin type) equal to that found in pregnancy urine. After the tumor had been removed, the active principle disappeared from the urine.

Assays with commercial preparations of chorionic gonadotrophin establish their minimal effective dose in the weaver-finch test at the range of 30 to 60 international units. Chorionic gonadotrophin, though principally a luteinizing preparation, possesses some FSH activity as well. The international unit, based on rat assays, is a measure of the composite gonadotrophin activity. The apparent difference between the international unit and the weaver-finch unit is most likely due to the dependence of the rat assay (used to establish the international unit) on the follicle-stimulating component of the preparation. The bird assay, on the other hand, depends solely on the luteinizing hormone contained therein.

These most recent experiences with the weaver finch in the field of gonadotrophin assay support the claim of Witschi for the luteinizing hormone specificity of the reaction and demonstrate the activity of human chorionic gonadotrophin in eliciting the weaver-finch feather response.

S. J. Segal

Laboratory of the Population Council, Rockefeller Institute, New York

References and Notes

1. The weaver finch is a small bird that naturally inhabits Africa, but it is readily available in the United States because large numbers are imported for sale to bird fanciers. The male bird assumes a bright yellow and black plumage at the onset of the breeding season and maintains this for several months. After molting, it dons the hen-type plumage which is characteristic of the female all year around. The hen plumage includes white breast feathers instead of the black ones found on the male during the breeding season. The plumage change in the male coincides with the cyclic change of the gonads from the quiescent to the breeding stage. Castrated males, however, continue to assume the breeding plumage rhythmically (*2*). This indicates that the feather pigmentation which occurs during the male phase of the plumage cycle is *not* controlled by gonadal androgens. On the other hand, injection of hypophysial suspensions into any bird, quiescent male or female, castrated or intact, induces the deposition of pigment in regenerating feathers. When this reaction is used as a bioassay, the test material is administered intramuscularly for 2 days; if it is positive, a black bar appears in the regenerating feathers during the period of threshold concentration. The response becomes apparent within 3 days after the injections. If the test is negative, entirely white feathers will regenerate [S. J. Segal, *Recent Progr. in Hormone Research* 12, 298 (1956)].
2. E. Witschi, *Wilson Bull.* 47, 177 (1935).
3. E. Witschi, *Mem. Soc. Endocrinol.* 4, 149 (1955).
4. These data were supplied by the Armour Laboratories, Chicago, Ill.
5. J. T. Grayhack *et al.*, *Bull. Johns Hopkins Hosp.* 96, 154 (1955); M. D. Chase, I. I. Geschwind, H. A. Bern, *Proc. Soc. Exptl. Biol. Med.* 94, 680 (1957).
6. S. J. Segal and E. Witschi, *J. Clin. Endocrinol. and Metabolism* 15, 880 (1955).

16 August 1957

Stability of Paramecin 34 at Different Temperatures and pH Values

A number of killer clones of *Paramecium aurelia* are known from the work of Sonneborn (*1*) and his associates. The stability of the killer substance from one of these clones, paramecin 51 (Pn 51) was studied in detail by van Wagtendonk (*2*). Recently Chen (*3*) has described two killer clones of *Paramecium bursaria,* Mi 34 and Ru 22. The killer substance of the clone Mi 34, paramecin 34 (Pn 34), is the subject of this present report (*4*).

Animals of the killer clone Mi 34 and of the sensitive clone Wu 67 were grown in a lettuce infusion inoculated with *Aerobacter cloacae* as described by Chen (*5*). The assay for Pn 34 was carried out essentially as described by van Wagtendonk and Zill (*2*) for Pn 51. The number of sensitive animals killed was found to be proportional to the amount of crude Pn 34 added, if at least a 50 percent excess of test animals was used.

Animal-free culture fluids from killer clone Mi 34 were obtained by filtration through cotton and subjected to a variety of temperatures. No attempt was made to control the *p*H of these fluids, which was in all cases close to 7.0. Immediately and at various time intervals samples were assayed for killer activity. At each temperature the activity of the first sample was arbitrarily defined as 100, and the subsequent activities are reported as a percentage thereof. Figure 1 shows the decay of Pn 34 activity at temperatures from 37° to 62°C. Each point represents the average of 2 to 20 determinations. At these temperatures Pn 34 was quite labile, and the decay followed the pattern of a first-order reaction. At temperatures below 37°C two complications appeared which require further investigation: (i) an apparent initial increase of killer activity over the zero time activity, similar to van Wagtendonk's (*2*) findings with Pn 51 at 20°C and (ii) a slight departure from a simple first-order decay reaction.

Animal-free killer culture fluid was obtained as described in the previous paragraph above, its *p*H was adjusted to the desired values with small amounts of 0.1*N* acid or base or 0.025*M* phosphate buffers, and the solution was kept at 37°C. The *p*H of the solutions remained constant to within 0.2 *p*H units for the duration of the experiments. At various times samples were assayed for Pn 34 activity. The samples at *p*H 2.7, 4.5, and 10.2 were neutralized before the assay, all other samples were used without adjusting the *p*H. The activity of the untreated killer culture fluid, diluted correspondingly, was arbitrarily defined as 100, and the other activities are reported as a percentage thereof. Fig. 2 shows the percentage killer activity left after 30 minutes at 37°C as a function of the *p*H of the solution.

A comparison of the data shown here for Pn 34 with van Wagtendonk's data for Pn 51 shows that the two substances have distinctly different *p*H optima, Pn

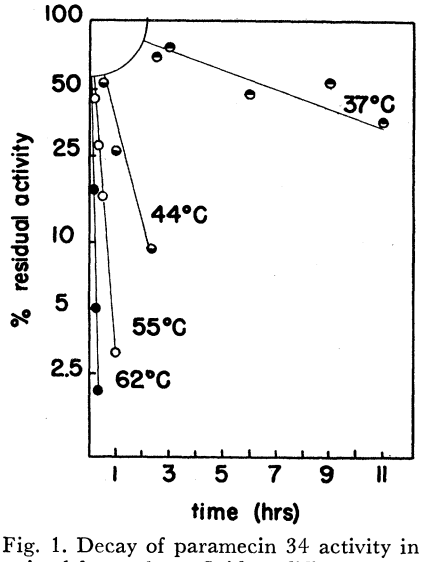

Fig. 1. Decay of paramecin 34 activity in animal-free culture fluid at different temperatures.