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.

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**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)].
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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

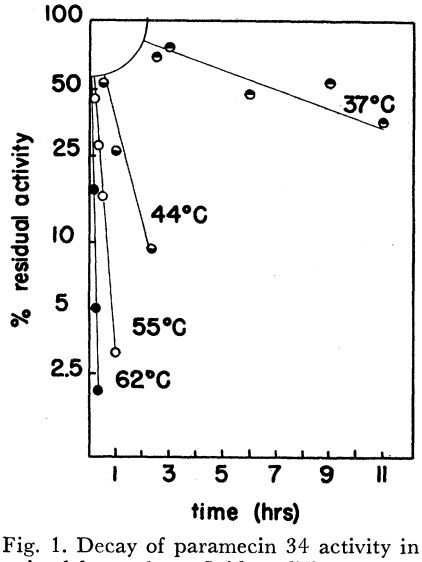


Fig. 1. Decay of paramecin 34 activity in animal-free culture fluid at different temperatures.

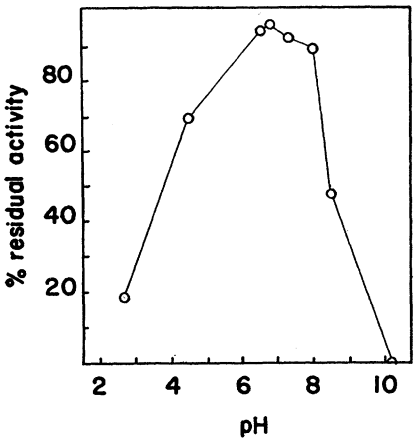


Fig. 2. Influence of *p*H on the stability of paramecin 34 at 37°C. The ordinate represents the residual activity after a 30-minute exposure to a particular *p*H, as explained in the text.

51 at 8.5 and Pn 34 at 6.8. At their respective *p*H optima both substances decay at similar rates.

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**References and Notes**

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5 August 1957

## Solubility of Carbon Dioxide in Body Fat

In recent studies (*1*) in this laboratory of the distribution of $CO_2$ in rats that had been exposed to atmospheres rich in this gas, it was found that a solubility coefficient for $CO_2$ in body fat was required for calculating the distribution of $CO_2$ in various tissues. The literature, however, contains little quantitative data on the subject (*2*). In 1907 Vernon (*3*) measured the $CO_2$ contained by samples of fats that had been equilibrated with air, but his data are not sufficient for calculating a solubility coefficient for the gas. Van Slyke, Sendroy, Hastings, and Neill (*4*) described solubility coefficients for $CO_2$ in whole blood, serum, and salt solutions, noting various factors which affected them. While these authors observed a higher solubility of $CO_2$ in lipemic sera, which they ascribed to the fat content, they did not measure the solubility of $CO_2$ in fat directly.

Vibrans (*5*) measured the solubility of $CO_2$ in cottonseed oil and lard, but no solubility coefficient for $CO_2$ has been determined for forms of mammalian body fat other than lard. I therefore undertook to determine such a number for the depot fat of three species: rat, dog, and man (*6*).

Depot fat was obtained as follows: Six 200-day-old male Wistar rats were killed by a blow on the head and exsanguinated, and fat from the perirenal, subcutaneous, and retroperitoneal areas was collected and pooled. This pool of tissue was mixed and divided into two lots. Perirenal and subcutaneous fat was taken from a single adult male mongrel dog 2 hours after it had been killed by exsanguination under anesthesia, and both types were combined in a single pool. Human fat was obtained 5 hours after death from the omentum of an 86-year-old woman. The fatty tissue collected was homogenized with sand in buffer solution of *p*H 4.0 at 30 to 35°C. One lot of rat fat was homogenized with buffer of *p*H 7.40. The containers were centrifuged for 30 minutes, and the clear upper layer of melted fat was removed with a pipette and stored at 5°C in a clean, tightly stoppered flask until it was used. Equilibration was carried out within 48 hours after the fat was obtained. No attempt was made to characterize the samples of fat chemically except as follows: water content was zero, for the samples failed to lose weight when they were stored in a desiccator for 48 hours over calcium chloride at room temperature; solubility in a 50/50 mixture of anhydrous ethyl ether and petroleum ether was complete; a melting point was determined for each sample.

Three- to five-milliliter samples of fat were equilibrated in flasks at 38°C for 2.5 to 5 hours. (Equilibration was complete after 1 hour.) A dry atmosphere containing 23.5 to 24.9 percent $CO_2$ was passed continuously through the flasks. Barometric pressure was noted, and the $pCO_2$ of the gas phase was corrected accordingly. After equilibration, samples were obtained for analysis by aspirating the fat anaerobically from the equilibration flask with a syringe and needle thrust through a rubber serum stopper. One-milliliter Van Slyke pipettes were filled with the liquid, equilibrated fat directly from the syringe without exposure to air. The specific gravity was determined for each type of fat studied. The volume of fat delivered by the pipette was actually less than 1 ml because of the high viscosity of the material. A suitable correction for fat left on the walls of the pipette was determined in each case on the basis of the specific gravity of the appropriate type of fat. This fat was then added anaerobically to 2.5 ml of previously extracted acidified water in the chamber of the Van Slyke manometric apparatus, and its $CO_2$ content was measured by the usual procedure.

The factors for the calculation of the total $CO_2$ content of samples given by Van Slyke and Sendroy (*7*) are based on the solubility of $CO_2$ in water. In our procedure the fluid phase was not homogeneous, but instead contained two compartments: 1 ml of fat and 2.5 ml of water, each having different solubility coefficients for $CO_2$. Therefore, new factors were calculated by obtaining a first approximation of the solubility coefficient for fat by use of the Van Slyke and Sendroy factors and then by recalculating the factors, using this number. The new factors were then applied to the original manometric readings.

The results obtained are shown in Table 1. The fats taken from the three species showed certain minor differences. The melting points for rat and human fat were almost identical. That for dog fat was considerably higher. This may have been an artifact, however, for this sample could have been contaminated, and the melting point was determined some time after the equilibration with $CO_2$ was carried out. Some change in the fat may have occurred during storage in the icebox. The specific gravity of the human fat appeared to be slightly lower than that of rat and dog fat.

Two samples of rat fat were studied. Sample A was extracted from adipose tissue with a buffer at *p*H 4.00, while sample B was extracted with a buffer at

Table 1. Solubility coefficients for $CO_2$ in depot fat at 38°C.

| Species | Melting point (°C) | Specific gravity | Number of samples equilibrated | $CO_2$ | | $f_{CO_2}$ |
|---|---|---|---|---|---|---|
| | | | | $cm^3/g$ | $cm^3/cm^3$ ($\alpha$) | |
| Dog | 30 | 0.9155 | 9 | 0.9405 ± 0.0140 | 0.8609 ± 0.0129 | 0.0556 |
| Rat (A) | 19 | 0.9154 | 9 | 0.9738 ± 0.0092 | 0.8917 ± 0.0077 | 0.0576 |
| Rat (B) | 19 | 0.9154 | 10 | 0.9813 ± 0.0089 | 0.8985 ± 0.0082 | 0.0578 |
| Human being | 18 | 0.9110 | 8 | 0.9306 ± 0.0483 | 0.8476 ± 0.0447 | 0.0550 |