manent sterility in *Habrobracon* feeding experiments (unpublished). This report (*1*) gives observations on the biological half-life, on the proportionate gross distribution, and on the egg radioactivity after these female braconid wasps have been fed Sr^{89}. The results differ, not only from findings on *Habrobracon* after P^{32} feedings (*2*), but also from strontium retention studies in vertebrates (*3, 4*).

Forty virgin females from wild-type stock 33 of *Habrobracon juglandis* [*Microbracon hebetor* (Say)] were starved at least 4 days, and each was weighed on a precision balance (Roller-Smith). They were then fed Sr^{89} citrate in a honey mixture at a level of 227 μc/g of mixture. With the stock solution of citrate available, this level resulted from mixing in equal proportions with honey. After feeding, each wasp was reweighed. Four that had not fed satisfactorily were discarded. Then, in order to obtain a measure of initial radioactivity for each animal, counts per second were obtained using a thin end-window Geiger tube with standard scaler. Subsequent maintenance followed standard practice in our laboratory. All wasps were provisioned with two host larvae (*Ephestia*) per day and maintained in individual Stender dishes at 30°C.

Twelve wasps were chosen at random to be followed in a study of total body radioactivity. The intact wasp (2 by 0.6 mm), periodically immobilized by brief cooling, can be subjected to Geiger-Müller counting. Thus counts per second were obtained for each animal of the sample 12 hours subsequent to feeding. The emissions were counted again at the end of 24 hours and at daily intervals for 11 days. After this time, the wasp radioactivity was barely above background. Figure 1 presents a summary of the results in net counts per second.

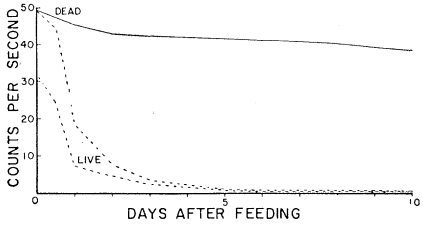

Fig. 1. The relative radioactivity of female *Habrobracon* fed Sr^{89} in honey mixture. The lower broken line represents average radioactivity for nine wasps that ingested the usual quantity, 0.2 to 0.3 mg of the mixture. The upper broken line shows the same for two females that ate 0.5 mg. The solid line, which extrapolates to an adequate representation of typical Sr^{89} decay, is taken to represent this phenomenon as measured in a dead wasp. The female expired shortly after its heavy feeding (0.5 mg).

In view of the physical half-life of 55 days, the biological half-life was achieved in the surprisingly short time of less than 1 day, which indicates very little retention. The dead animal carried for comparison demonstrates the loss in radioactivity owing to isotope decay only (Fig. 1). This picture is quite different from that in vertebrates, in which more than half of a dose may persist after 9 days (*4*) and in which the rate of elimination can be less than the rate of radioactive decay (*5*).

A further point brought out by this experiment is that differences in wasp radioactivity following heavy and average feedings are not significant after the biological half-life has been exceeded. Even at the end of the first day, the standard error of the mean for the animals from average feedings is 9.97 counts per second. On the second day, the range of the groups includes the mean value for the high feedings. Ultimately, as is shown by Fig. 1, the curves converge when the means become identical.

The remaining 36 animals were sacrificed in groups of four by transection at the petiole, so that the anteriors and posteriors could be counted separately (sacrifice schedule: days 2, 4, 6, 7, 8, 9, 10, 11, and 12). During the first week after feeding, it was evident that more than 90 percent of the radioactivity that was demonstrable for each female came from the abdomen. Indeed, in the majority of cases, 97 to 99 percent of the radioactivity was shown to be abdominal. In the second week, when radioactivity of the entire animal was barely above background, abdominal radioactivity fell off only slightly. On the 12th day, the abdomens held 86 to 92 percent of total radioactivity. The abdomen contains most of the digestive tract and all the organs of excretion and reproduction.

Eggs laid by the wasps of the transection experiment were collected until the day of sacrifice, and their radioactivity was determined. Eggs laid on the first 3 days were slightly radioactive, an indication that at least some Sr^{89} gets into the physiological interior of fed females. Eggs laid on the third day were barely above background radioactivity. Those laid on subsequent days were not demonstrably radioactive. The average net counts per second per egg were 0.0094 on day 1, 0.0040 on day 2, and 0.0007 on day 3.

When these results were compared with the radioactivity of the ovipositing females, it was found that little more than 0.03 percent of the radioactive material lost from the animal was lost by way of the eggs. This is consistent with the indications from the biological half-life that egestion of feces and excretion plays the important role for this isotope, a finding quite different from that with P^{32}, where the majority of the fed isotope is incorporated into the eggs and eliminated by this route (*2*).

These discoveries concerning the fate of radiostrontium explained a perplexity that is met in autoradiographic technique. In direct contrast to the results obtained when autoradiographs are made after radiophosphorus feedings (*2*), radiostrontium shows up only in the oocytes and not even there if more than 2 days have elapsed since its ingestion. Apparently adult insects have no tissue functionally analogous to bone in a biochemical sense. In vertebrates, the presence of bone with its propensity for fixing the alkaline earth elements is responsible for the retention of strontium. On the other hand, it is well established that the radioactivity of soft tissue of vertebrates is negligible after doses of Sr^{89} (*3, 4*). Our results are essentially a demonstration of this latter point for an adult insect.

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Alkaline Phosphatase in Kidneys of Aglomerular Fish

In a recent issue of *Science*, I discussed the implications of the presence of alkaline phosphatase in the aglomerular renal tubule (*1*). This discussion was based on unpublished observations by Danielli and Lorch and on my own experience with the kidney of *Opsanus tau*. Since the publication of this note, my attention has been called to an earlier paper by Browne and associates (*2*), who reported the presence of this enzyme in the tubules of a number of teleosts, including three aglomerular species, and in particular, *Opsanus tau*. From their observations, these authors drew essentially the same conclusions about alkaline phosphatase and tubular function as I did. Priority on these points therefore rests with them.

Our conclusions with respect to the significance attached to previous reports of negative results differ. Browne and associates believed that slight variations

of fixing technique probably caused these and thereby apparently closed the matter. However, there are now, including those mentioned in our two papers, sufficient observations from different sources to conclude that kidney tubular alkaline phosphatase may be markedly variable in fish and reptile species. Further work is needed to define the conditions that govern these changes and to determine the consequent functional alterations.

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Application of Paper Chromatography to Taxonomic Studies

The preliminary work of Buzzati-Traverso and Rechnitzer (*1*) suggested that the simple method of squashing fresh tissue on filter paper, followed by one-dimensional chromatographic separation of ninhydrin-positive and ultraviolet-fluorescent substances, could yield results of value in taxonomic and population-genetic studies. These expectations have been amply fulfilled in an extensive investigation of the dipteran family Drosophilidae (*2*).

The method as previously applied has, however, suffered from limitations imposed by the inadequate separation of complex mixtures that is afforded by one-dimensional chromatography. In *Drosophila melanogaster*, for example, differences between males and females with respect to ninhydrin-positive materials are readily demonstrable by means of two-dimensional chromatography (Figs. 1 and 2). The most striking difference is the presence of a peptide in males that is absent in females, but quantitative differences exist as well (*3*). When, however, the same solvents are used separately in the development of one-dimensional chromatograms, the differences either fail to be disclosed (in the case of the butanol, acetic acid, and water mixture) or spurious differences are observed (in the case of 80-percent aqueous phenol).

These misleading observations are the result of a number of factors. In the first place, the spots observed on one-dimensional chromatograms frequently consist

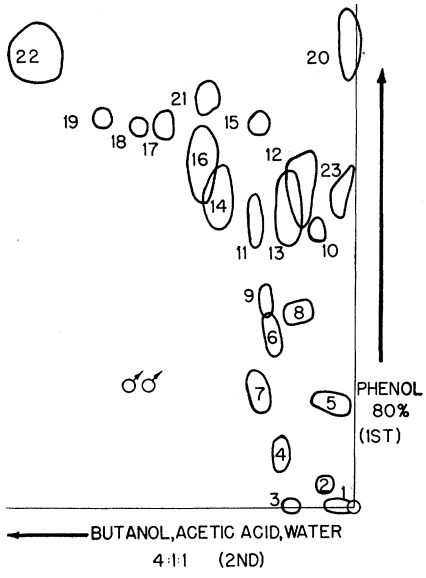

Fig. 1. Two-dimensional chromatogram of free ninhydrin-positive substances in ten decapitated *D. melanogaster* males that were squashed directly on Whatman No. 1 filter paper. First solvent: 80-percent aqueous phenol for 20 hours at 25°C. Second solvent: *n*-butanol, glacial acetic acid, and distilled water (4 to 1 to 1 by volume) for 18 hours at 25°C. Identity of spots: *1*, unknown; *2*, pupine (?); *3*, unknown; *4*, aspartic acid; *5*, cystine; *6*, serine; *7*, glutamic acid; *8*, taurine; *9*, glycine; *10*, lysine; *11*, threonine; *12*, histidine and/or arginine; *13*, glutamine; *14*, α-alanine; *15*, methionine; *16*, β-alanine; *17*, tryptophan; *18*, valine; *19*, norvaline; *20*, front peptide (?); *21*, proline; *22*, leucines; *23*, sex peptide (specific to males).

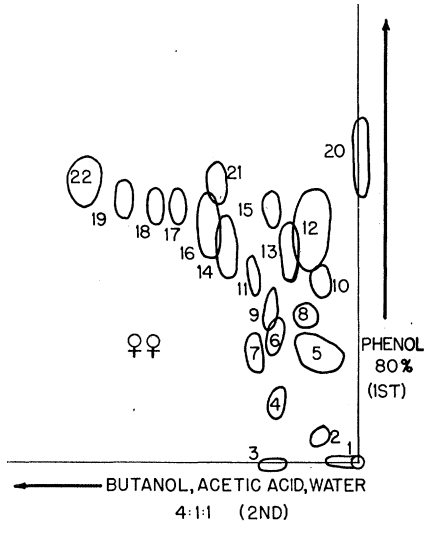

Fig. 2. Two-dimensional chromatogram of free ninhydrin-positive substances in eight decapitated *D. melanogaster* females. Development and identity were the same as in Fig. 1. Note absence of sex peptide, spot *23*.

of two or more substances with similar R_f values, and the differences between the sexes are thus obscured. Further, inspection of Figs. 1 and 2 discloses a systematic depression of R_f values in females in the dimension that is developed with phenol. In one-dimensional chromatograms developed with this solvent, this depression of R_f values results in a compaction of spots and an apparent reduction in the number of ninhydrin-positive substances in females as compared with the number in males.

These observations (*4*) illustrate the obvious advantages of two-dimensional chromatography and suggest its more extensive use in the application of paper chromatography to problems of taxonomy and population genetics. They also suggest that the establishment of the identity of or difference between substances in different species or populations should not depend only on R_f values and such gross observations as color of spots but should also include qualitative identification by means of more extensive chromatographic and chemical procedures whenever possible. Quantitative measurements as well as qualitative identifications would be highly desirable.

Thus, an examination of the distribution of individual, identified substances among species or populations would be preferable to that of over-all chromatographic patterns. As a first approach, hierarchies of chromatographic similarities and differences should be useful in the construction of taxonomic categories, although a more refined approach might be provided by methods of multivariate analysis (*5*). Properly used, chromatographic methods should come to occupy a position in modern taxonomy similar to that occupied by serologic methods.

A more complete account of the methods of chromatography and identification employed in this work, as well as an analysis of R_f values, will be published elsewhere.

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22 August 1955