cans (*6*) were also present in or on the surface of modern clam shrimp ponds in the sampled area. In this instance, as with the conchostracans, it is clear that habitat-preference and adaptation which were established or operative in Leonardian time still persist.

While the fossil collections have yet to be thoroughly analyzed for clam shrimp populations, certain observations, based on field and laboratory notes are possible at present. A one-species spread such as that for *Cyzicus mexicanus* seems, at any given time, to have characterized only portions of the Leonardian outcrop belt in Kansas and Oklahoma. Thus, one of the oldest clam shrimp zones, some 10 feet above the Annelly gypsum, contained three different generic types: pemphicycliids (bearing a tubercle or spine on the initial valve), typical estheriids (lacking valve structures), and, at one Oklahoma locality, leaiid conchostracans (bearing two ribs on the valve). Or, in Kansas localities, three related but distinct genera were found in contemporaneous beds: *Pemphicyclus* (initial valve with central minute tubercle), *Gabonestheria* (large anterodorsal conical spine on initial valve), and *Curvacornutus* (with large anterodorsal, looped or hooked spine on initial valve). Multiple instances of this kind of differentiation (speciation) in contemporaneous ponds could be cited (*7*).

Thus, the fossil record in the mapped area (Fig. 1) indicates a greater incidence of genetic variability at specific times and at several different times of clam shrimp appearance during the Wellington. In turn, this refers to a more frequent geographic fractionation of the common gene pool with attendant reproductive isolation and speciation in Leonardian situations than is found in modern ponds in the sampled area. The evaporites noted earlier denote a more arid climate with consequently greater alternation of drying and wetting. This might well be a critical factor in the "more frequent geographic fractionation" referred to above (*8*).

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2. Constructed farm ponds are not included in this sample.
3. Data were obtained from the U.S. Weather Bureau, Wichita, Kan., for the years 1957 through June 1960. The belt of investigation lies in and occupies most of the 30–35 in. rainfall zone.
4. The spotty distribution of *Cyzicus mexicanus* in the sampled belt recorded during the 1960 sampling confirms field observations made during the less intense collecting of the summers of 1958 and 1959. During the 1960 field season, some areas that contained no clam shrimp ponds in June or July were re-explored during the early weeks of August. Of 35 such ponds sampled, clam shrimps were found in four, or in 11.4 percent of the total sample. This figure confirms the 12 percent over-all average (Table 1). Thus, while a few more scattered clam shrimp ponds might be located by continued visitations to the same areas, it is unlikely that the over-all average will be importantly increased.
5. Data on faunal associates, multiple generations per season, special cases, and so forth, are too detailed for inclusion and analysis here.
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9. P. Tasch, "Newly discovered conchostracan-bearing beds of the Ninnescah formation of Kansas," Geological Society of America program (Pittsburgh, Pa., 1959), p. 12 (abstr).

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Metabolism of Adrenaline after Blockade of Monoamine Oxidase and Catechol-O-methyltransferase

Abstracts. Experiments in cats infused with 5 μmole of *dl*-adrenaline-2-C^{14} showed that blockade of either monoamine oxidase or catechol-O-methyltransferase is largely compensated for by the activity of the intact enzyme system; combined blockade of both enzyme systems results in the formation of a new adrenaline catabolite and in the increased production of acidic, mainly conjugated, catabolites, the identity of which remains to be established.

Previous studies on the metabolism of adrenaline and noradrenaline have shown these hormones to be inactivated mainly by oxidative deamination and O-methylation (*1*, *2*). In order to evaluate more fully the role of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) in the inactivation of adrenaline and noradrenaline, the work described below was undertaken.

Cats anesthetized with Nembutal were infused over a 5-minute period through a cannula in a femoral vein with 5 μmole of *dl*-adrenaline-2-C^{14} (specific activity, 1.25 μc/μmole) dissolved in physiological saline. The animals were killed 5 minutes after the infusion. Blood, heart, liver, and kidneys were removed, homogenized in 10 percent trichloroacetic acid, and stored at −15°C until assayed. After the homogenates were filtered on a suction flask, the residue was extracted three times more by homogenizing in 5 percent trichloroacetic acid and filtering. The combined filtrates were then extracted three times with ether to remove the acid. The residual ether was evaporated *in vacuo*, and a sample of the aqueous solution was taken for determination of total radioactivity. The remainder was concentrated in a rotating flash evaporator at 35°C, and the *p*H of the concentrate was adjusted to 6.8.

The radioactive products which appeared in the blood and tissues after the infusion of *dl*-adrenaline-2-C^{14} were separated by a modification of the procedure previously described for urine (*2*). The metabolic pattern was much more complex in blood and tissues than in urine, and resolution of the various fractions was not as clearly defined.

The various catabolites were essentially identified by paper chromatographic analysis of the various fractions, with and without previous acid or enzymatic hydrolysis, with three different solvent systems: butanol saturated with 1*N* HCl; isopropanol, ammonia, and water (8:1:1); and butanol, acetic acid, and water (4:1:1).

Table 1 shows the pattern of metabolism of *dl*-adrenaline-2-C^{14} in controls; in cats after treatment with iproniazid (*3*) (100 mg/kg, intraperitoneally, 24 hours and 4 hours prior to the infusion); in cats after treatment with pyrogallol (*4*) (150 mg in 30 ml of physiological saline, intravenously) immediately before the infusion; and in cats after combined treatment with iproniazid and pyrogallol (same doses). It should be mentioned that at this time it is not our intent to present mathematically significant data on the distribution of adrenaline and its catabolites in the various tissues, but to present semiquantitatively the general aspects of adrenaline metabolism to serve as an orientation for future studies. The data in Table 1 are discussed in the sense that they show the major metabolic changes undergone by adrenaline and the effects of inhibition of the enzyme systems involved.

Adrenaline was found to disappear very rapidly from the blood and, except in one instance, it constituted only a small fraction of the total radioactivity recovered. The highest concentrations of free adrenaline occurred in the liver. Metadrenaline was by far the most important basic fraction. In some cases an additional, hitherto unidentified, basic catabolite was found having an R_F value slightly less than that of adrenaline in butanol and 1*N* HCl.

Among the neutral and acidic catabolites, peak 1 of Table 1 has been tentatively identified as a mixture of 3-methoxy-4-hydroxyphenylethylglycol, 3,4-dihydroxyphenylethylglycol, and possibly conjugates of metadrenaline and adrenaline; peak 2 is still unidentified; peak 3 is 3-methoxy-4-hydroxymandelic acid; and peak 4 is 3,4-dihydroxymandelic acid. Peak 5 is a mixture of various fractions; there is evidence that

Table 1. Distribution of radioactivity after intravenous infusion of *dl*-adrenaline-2-C^{14}. Figures are percentages of total radioactivity isolated from each tissue. Peak 1 represents 3-methoxy-4-hydroxyphenylethylglycol, 3,4-dihydroxyphenylethylglycol, and possibly conjugates of adrenaline and metadrenaline. Peak 2 represents an unidentified compound. Peak 5 represents a mixture of conjugates of both the glycols and both the mandelic acids.

Treatment	Control			Iproniazid		Pyrogallol			Pyrogallol and iproniazid		
					Blood						
Adrenaline	4	*	3	1	1	2	2	*	1	54	8
Metadrenaline	23	*	18	29	15	1	*	*	1	7	2
Peak 1	12	13	18	34	43	15	5	15	14	5	10
Peak 2	7	9	8	7	7	7	20	26	18	5	13
MOMA	11	13	7	4	1	4	3	9	3	2	3
DOMA	1	*	*	*	1	3	5	13	10	1	6
Peak 5	27	28	24	18	10	48	40	32	31	7	32
					Heart						
Adrenaline	9	1	7	1	2	3	2	1	*	37	7
Metadrenaline	44	15	22	56	35	2	*	1	*	5	2
Peak 1	4	11	17	6	8	12	7	17	14	7	17
Peak 2	*	11	3	6	7	7	7	26	9	2	21
MOMA	3	13	3	3	3	4	7	6	10	2	7
DOMA	*	*	3	4	2	7	13	4	9	2	8
Peak 5	2	28	18	11	12	26	44	27	33	10	30
					Liver						
Adrenaline	1	6	9	16	19	21	13	16	51	56	49
Metadrenaline	27	45	44	64	40	9	2	9	14	3	5
Peak 1	30	14	16	1	*	1	3	3	4	16	3
Peak 2	2	1	*	10	12	15	8	10	1	4	2
MOMA	11	7	4	*	*	1	1	3	*	4	1
DOMA	12	*	4	*	*	11	8	8	*	5	*
Peak 5	24	18	14	*	*	2	*	21	1	13	2
					Kidney						
Adrenaline	2	1	4	1	1	6	9	1	4	46	6
Metadrenaline	36	36	14	76	52	4	1	1	2	7	1
Peak 1	7	8	34	8	14	36	*	23	18	3	16
Peak 2	2	*	*	3	2	2	30	28	22	4	18
MOMA	7	4	3	2	1	2	*	8	3	3	5
DOMA	*	4	*	*	2	1	*	5	4	*	5
Peak 5	23	41	20	*	8	19	*	30	26	3	30

* Less than 1 percent.

they are conjugates of both the glycols, and both the mandelic acids just mentioned.

In the untreated animals more than 70 percent of the recovered radioactivity in the blood was found in the neutral and acidic fractions; in the heart, liver, and kidneys, 50 percent of the recovered radioactivity was found in these fractions. In the blood, kidney, and heart, 60 to 70 percent of the radioactivity in the acidic and neutral fractions was accounted for as 3-methoxy-4- and 3,4-dihydroxyphenylethylglycol, and the mixture of conjugates. The two mandelic acids, and the unidentified compound in peak 2 represent the remaining radioactivity.

Treatment with iproniazid or pyrogallol or both increased the amount of total radioactivity recovered in the blood and heart but did not noticeably alter the total radioactivity in the liver and kidneys.

The principal effect of treatment with iproniazid was to increase the amounts of metadrenaline in all of the tissues; only in the liver was there a marked increase in the amounts of adrenaline. Concomitantly, the amounts of 3-methoxy-4- and 3,4-hydroxymandelic acid and of the mixture of conjugates were decreased. It is noteworthy that treatment with iproniazid did not significantly influence peak 2, which suggests that the substance corresponding with this peak is not a product of monoamine oxidase activity.

Treatment with pyrogallol markedly decreased the metadrenaline content of the blood and tissues. The adrenaline contents of the kidneys and liver were increased—in the latter, to about the same extent as that after treatment with iproniazid. The mixture of the conjugates is markedly enhanced in the blood and heart but, on the average, is lowered in the liver and kidneys. The mixture of 3-methoxy-4- and 3,4-dihydroxyphenylethylglycol was decreased in the liver but was not significantly different from the controls in the heart, blood, and kidneys. The fact that peak 2 was found unchanged or increased in these circumstances suggests that it is also not a product of catechol-O-methyltransferase activity.

Combined treatment with iproniazid plus pyrogallol provoked a very marked decrease of methylated catabolites. The amounts of adrenaline found in the liver were markedly higher than in the controls; in the other tissues, only in one of the three instances was the level of adrenaline highly elevated. The metabolism of *dl*-adrenaline-2-C^{14} mainly consisted in the increased production of the catabolite corresponding with peak 2 and in the formation of a new unidentified basic catabolite with an R_F value slightly less than that of adrenaline in butanol and 1*N* HCl. This alternate catabolite occurs mainly in the liver, where it may represent as much as 35 percent of the total radioactivity. In one animal this pathway also appeared to be the most important in the heart and kidneys. In the two other animals, however, this did not occur, and the metabolism of adrenaline apparently consisted in the enhanced production of acidic, mainly conjugated, catabolites, the identity of which remains to be established.

The results of the studies reported here indicate two major alternative pathways—oxidative deamination and O-methylation—and two minor pathways—conjugation and an unidentified reaction—for the metabolism of adrenaline in the cat. In the heart, both monoamine oxidase and catechol-O-methyltransferase appear to be present in sufficient amounts to compensate for decreased activity on the part of one or the other of the enzyme systems. In the liver, inhibition of either one of the enzymes results in a decreased rate of metabolism of adrenaline; however, as in the heart, inhibition of either monoamine oxidase or catechol-O-methyltransferase is largely compensated for by the activity of the other enzymes capable of metabolizing adrenaline. In the kidney, inhibition of monoamine oxidase is completely compensated for by catechol-O-methyltransferase activity, whereas inhibition of catechol-O-methyltransferase is not completely, but is to a very large extent, compensated for by the activity of monoamine oxidase and other enzymes (*5*).

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