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Constitutive Enzymes of the Developing Chick Embryo: Adenosine Deaminase

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The study of enzyme activities in developing tissues yields information only about the overall potential metabolic pattern and gives no clue to the nature of the chemical or biological stimulus which promotes the change in the differential rate of accumulation of a certain enzyme. If a specific stimulus to promote enzyme synthesis could be found, it would be possible to measure the capacity for enzyme synthesis of an embryonic tissue at any stage of its development. It is this capacity of embryonic cells to be controlled by their environment, at different stages of development, that may influence the cells' ultimate function. An attractive way of viewing the complex processes of differentiation is as 'a series of mutually dependent endogenously induced enzyme formations' (Rusch, 1954). The phenomenon of adaptive increase in enzyme activity is well known in bacteria (e.g. Monod, 1947; Spiegelman, 1948), as are increases in the activity of mammalian enzymes due to certain non-specific stimuli, such as the variation of dietary protein (e.g. Mandelstam & Yudkin, 1952), changes of altitude (Tappan, Reynafarje D., Potter & Hurtado, 1957), hormones or disease (Knox, Auerbach & Lin, 1956). However, relatively few cases of substrate-induced enzyme adaptations, other than that of tryptophan peroxidase (e.g. Knox, 1951), have been found in mammals.

If the development of the ability to produce certain enzymes during normal growth is due to a type of enzymic adaptation (otherwise known as enzyme induction) it might be expected that this capacity (with respect to any particular enzyme) could be prematurely stimulated by exposure of embryonic cells to an excess of substrate.

In the chick embryo only three cases of substrate-induced enzyme adaptation have so far been reported (Gordon & Roder, 1953; Gordon, 1956; Roeder, 1957). Arginase was found to be increased by 30–143% in the whole chick embryo during 5–14 days of incubation by injecting arginine into the egg (Roeder, 1957). Tryptophan peroxidase was increased tenfold in the developing chick embryo by injections of tryptophan during the third to ninth days of incubation (Gordon, 1956). Gordon & Roder (1953) found that adenosine-deaminase activity increased eightfold in whole chick embryos at 8 days of incubation after the eggs had been injected with adenosine 24 hr. earlier. These authors also found a sevenfold increase of enzyme activity in the liver at 14 days of incubation after seven consecutive daily injections of small amounts of adenosine.

No adaptive increase in lactic-dehydrogenase activities in the liver of the chick embryo was observed after injection of sodium pyruvate or lactate into the egg at various stages of development; similarly no adaptive increase in liver glutaminase was observed after injections of glutamine (J. B. Solomon, unpublished results). It was concluded that lactic dehydrogenase and glutaminase were constitutive enzymes, in that they are normally produced by the cell and are not susceptible to stimulation by the presence of their substrates. In view of the paucity of examples of enzyme induction in any type of embryo (Markert, 1958), we have attempted to confirm the results of Gordon & Roder (1953) on adenosine deaminase.

A preliminary report of these results has already been published (Palmer & Solomon, 1959).

EXPERIMENTAL

Tissues. Eggs from White Leghorn fowls (Appleby Farm Ltd., Ashford, Kent) were incubated at 37° in a Westernette incubator. The method of removal of whole chick embryos and yolk sacs from the eggs has previously been described (Solomon, 1958). Livers were obtained from White Leghorn chicks and cockerels which had been hatched from these eggs, except for some livers from 34-week-old Rhode-Island Red × Light Sussex cockerels.

Injections of adenosine. Adenosine (L. Light and Co. Ltd.) was recrystallized twice from hot water and made up as a sterile solution (16.7 mg./ml.) in 0.9% NaCl. A batch of eggs which had been incubated from 1 to 10 days was divided into a control group (about 15 eggs) and an experimental group (25 eggs). The latter group of eggs received sterile 0.9% NaCl containing 10, 20 or 25 mg. of adenosine, injected into the air sac by the technique of Gordon & Roder (1953). The control group was injected with the corresponding volumes of sterile 0.9% NaCl. The eggs were then sealed with paraffin wax and returned to the incubator for 18–22 hr. Tissues were removed from the experimental and control eggs by the method previously described (Solomon, 1958). Tissues from the control eggs were pooled for the control homogenate and tissues from all the experimental eggs were pooled for the one experimental homogenate.

Homogenates. Tissues were homogenized in 0.2M-phosphate (Na_2HPO_4 – NaH_2PO_4) buffer, pH 7.0, in a glass homogenizer with a Teflon pestle. Homogenates of whole chick embryos and yolk sacs were then centrifuged at 1000 *g* for 10 min. (Gordon & Roder, 1953) and the supernatant was poured off and assayed for adenosine-deaminase activity. Liver homogenates were not centrifuged except when they were to be used in a spectrophotometric method of estimation. Homogenate concentrations were, for whole embryo or yolk sac, 1 g. wet wt./5 ml., and, for liver, 1 g. wet wt./15 ml. of phosphate buffer.

Measurement of adenosine deaminase by Kalckar's method. The method of Kalckar (1944, 1947) as described by Gordon & Roder (1953) was used. This method is based on the decrease in absorption at 265 m μ due to the conversion of adenosine into inosine by adenosine deaminase. Adenosine (0.3 ml. of a 4 mg./ml. solution) in 0.2M-phosphate buffer, pH 7.0, was added to 0.2 ml. of centrifuged homogenate and the mixture incubated at 38° for 30–60 min. Samples of centrifuged homogenate (0.2 ml.) from control embryos (four tubes) and experimental embryos (four tubes) together with the adenosine solution (0.3 ml.) were incubated at 38°. Similar samples but without adenosine were used as blanks. The reaction was stopped by the addition of 1.5 ml. of 0.5M-perchloric acid, the mixture was centrifuged for 5 min. and a sample of the supernatant diluted 50 times with water. The absorption of the diluted supernatant at 265 m μ was corrected by subtracting that at 290 m μ from all solutions. No proportionality between concentration of homogenate and activity was found when centrifuged homogenates were assayed by this method. Activities when 0.2 ml. of homogenate was used were about one-twentieth of the value obtained by the ammonia method. A modification of Kalckar's method as used by Schneider & Hogeboom (1952) was then used. Two samples (0.05 ml.) of centrifuged homogenate (24 000 *g* for 30 min.) were each diluted to 3 ml. with 0.2M-phosphate buffer, pH 7.0, and

the diluted homogenates were poured into 1 cm. silica cells. Initial and final measurements were made in a spectrophotometer (Unicam SP. 500) at room temperature (20°). The extinction reading for the blank was set at 0.2 and at zero time adenosine (0.01 ml. of a 6 mg./ml. solution) was added to the other cuvette and mixed by stirring with a spatulate glass rod. Readings of *E* at 265 and 290 m μ were made against the blank. The two solutions were then quickly transferred to stoppered tubes and incubated at 38°, with shaking, for 1 hr. After cooling to 20° the extinctions were then read again in the same way. The difference between the corrected extinctions ($E_{265 \text{ m}\mu} - E_{290 \text{ m}\mu}$) before and after incubation gave the amount of adenosine deaminated.

Measurement of adenosine deaminase by the determination of ammonia. Samples (0.5 ml.) of homogenate were incubated with adenosine (0.15 ml. of a 4 mg./ml. solution) in 0.2M-phosphate buffer, pH 7.0, for 30–60 min. The reaction was stopped by the addition of 15% (w/v) trichloroacetic acid solution (0.5 ml.). The ammonia was separated from the homogenate by the Conway (1947) microdiffusion technique and measured by a colorimetric method based on the phenol-hypochlorite reaction (Russell, 1944). Quadruplicate ammonia assays were made on the homogenate both with and without adenosine. The difference in amount of ammonia was then due to adenosine-deaminase activity. When the adenosine-deaminase activities of homogenates of embryos (about 15) receiving doses of 0.9% NaCl and embryos (about 25) receiving doses of adenosine were being compared, the one type of tissue (whole embryo, yolk sac or liver) of each group was pooled for one homogenate. The enzyme activity of each homogenate was then determined by quadruplicate assay as described above; the total number of determinations for two homogenates was 16. The coefficient of variation of the mean (amount of ammonia evolved) of any one group of quadruplicate assays was within the range ± 0.7 – 9.0% . Activity was proportional to the amount of homogenate up to 0.6 ml. of homogenate of chick embryo or yolk sac.

Activity units. After subtraction of the amount of ammonia evolved by a homogenate without adenosine from the amount of ammonia evolved in the presence of adenosine the difference was multiplied by 15.7 to express the units of activity in terms of rate of breakdown of adenosine. One unit of activity of adenosine deaminase is defined as the amount required to deaminate 1 μg . of adenosine in 30 min. at 38°.

Protein determination. The method of Sutherland, Cori, Haynes & Olsen (1949) was used. Bovine-plasma albumin (The Armour Laboratories) dissolved in 0.1M-phosphate buffer, pH 9.0, was used as a standard. Estimations were made within the range 10–120 μg . of albumin in 3.5 ml.

Statistical treatment. As the blank values in the ammonia method are about one-half of the values obtained in the presence of adenosine the *t* test has been used to test for significance of activity. Only values which are significant at the level *P* 0.05 have been used.

The significance of any increase in activity of the experimental homogenate above that of the control was assessed by calculating the standard error of the difference of the means (four determinations on each homogenate) and applying the *t* test. Only increases which are significant at the level *P* 0.05 are given in the tables.

RESULTS

The toxicity of adenosine to chick embryos varied considerably during development. It has been necessary to administer large doses of adenosine (25 mg.) to produce any significant increase of adenosine deaminase in the embryos, and the administration of this large dose has been possible only during the 6–8-day period of incubation. The large volume (1.5 ml.) of 0.9 % NaCl required to dissolve 25 mg. of adenosine practically filled the air sac; more concentrated solutions of adenosine started to crystallize at 37°. It was possible to inject eggs at 1–8 days of incubation with 20 mg. of adenosine in 1.2 ml. of 0.9 % NaCl without causing more than 30–50 % deaths. This is an extension of Gordon & Roder's work (1953) as these authors did not inject the embryos before the seventh day of incubation. The mean percentage survival figures are given in Table 1. The mean percentage survival figures for the controls ranged from 90 to 100 %.

Gordon & Roder (1953) obtained the greatest increase of adenosine-deaminase activity in the whole chick embryo by the injection of 25 mg. of adenosine into the egg at 7 days of incubation. Accordingly, we first tested for enzyme adaptation in eggs at this stage of development with the spectrophotometric method for adenosine deaminase used by Gordon & Roder (1953). No difference in

activity between the experimental whole-embryo or liver homogenates and control homogenates was observed. However, the absolute units of activity were at most one-twentieth of those obtained by Gordon & Roder (1953) with the same method. When the modified spectrophotometric method due to Schneider & Hogeboom (1952) was used the values of adenosine-deaminase activity were of the same order as those obtained by the ammonia method (Table 2). To obtain good reproducibility by the modified spectrophotometric method it was necessary first to remove particulate material by high-speed centrifuging of the homogenates (24 000 *g* for 30 min.). This centrifuging should remove only up to 20 % of the total adenosine-deaminase activity of a homogenate as, when the conventional cell-fractionation technique of Schneider & Hogeboom (1950) was used with a liver of a 34-week-old cockerel, 80 % of the total activity remained in the supernatant fraction (Table 3). The activity of the enzyme in this fraction was optimum at pH 7.0.

Adenosine-deaminase activity of untreated embryos

The adenosine deaminase/mg. of protein in the whole chick embryo remains constant at 7.7 ± 0.6 (23 results) activity units during 3–14 days of incubation (Fig. 1). The activity of homogenates of 2-day-old chick embryo at 1.0 unit is significantly lower (P 0.01) than that of embryos at later stages of development and it is probable that adenosine-deaminase activity has just appeared in the chick embryo at 2 days of incubation (24 hr. after the appearance of the primitive streak). The scattered values of adenosine-deaminase activity/mg. of protein in the developing yolk sac from 2 to 9 days of incubation have a mean value at 9.1 ± 1.2 (12 results) activity units. Liver-adenosine-deaminase activity has a mean value of 8.6 ± 0.8 (26 results) activity units/mg. of protein from 7 days of incubation to 34 weeks after hatching.

Table 1. *Toxicity of single doses of adenosine at different stages of development of the chick embryo*

Incubation time when injected (days)	No. of embryos	Dose of adenosine (mg.)	Mean survival (%)
1–2	126	20	50
3–7	189	20	70
4–6	176	25	40
7–8	319	25	60
9–13	27	25	0

Table 2. *Comparison of adenosine-deaminase activities obtained by the ammonia and the modified spectrophotometric methods*

Homogenate	Incubation time when injected (days)	Dose of adenosine (mg.)	Adenosine-deaminase activity (units/mg. of protein)			
			Ammonia method		Spectrophotometric method	
			Control	Experimental	Control	Experimental
Yolk sac	1	20	2.8	3.4	6.0	7.8
	3	—	10.6	—	11.0	—
	4	20	14.5	16.2	16.7	16.4
Chick embryo	6	25	7.4	9.7	4.5	5.4
	7	25	—	—	10.6	14.2
	8	25	9.0	8.6	11.7	8.5
Livers	9-day chick	—	8.6	—	5.6	—
	11-day chick	—	6.6	—	4.6	—
	38-week cockerel	—	12.0	—	12.5	—

Table 3. *Distribution of adenosine deaminase (ammonia method) among liver-cell fractions of a 34-week-old White Leghorn cockerel*

Cell fraction	Adenosine deaminase (% of total)	Adenosine-deaminase activity (units/mg. of protein)
Nuclei	8.3	6.3
Mitochondria	2.8	8.8
Microsomes	8.6	8.2
Supernatant	80.3	17.5

Fig. 1. Adenosine-deaminase activity/mg. of protein in centrifuged homogenates of whole chick embryos during early development.

Adenosine-deaminase activity of embryos dosed with adenosine

The injection of 20 mg. of adenosine into the air sacs of eggs incubated for 1–7 days produced, in two cases, an increase of enzymic activity (37 %) in the centrifuged homogenates of the whole embryos removed 24 hr. after injection (Table 4). With the embryos receiving 25 mg. of adenosine at 6, 7 and 8 days of incubation, four out of eight centrifuged homogenates at 24 hr. after injection showed 29–108 % increases of adenosine-deaminase activity (Table 4). However, increased activities were not obtained in the other experiments and the mean values of all the control and experimental adenosine-deaminase activities were not significantly different for either dose.

Adenosine deaminase was also measured in the yolk sacs of embryos dosed with 20 mg. of adenosine between 1 and 8 days of incubation. It was thought that the yolk sac, which is known to behave as a ‘transitory liver’ with respect to glycogen storage (Bernard, 1872) and to activities of glutamic, lactic and malic dehydrogenases

(Solomon, 1957, 1958) during development, might be capable of increasing its adenosine-deaminase activity. However, five out of 11 adenosine-deaminase activities in the yolk sacs of the dosed embryos were decreased by 31–39 %, although the mean values of all the control and experimental activities were the same (Table 5).

Table 4. *Adenosine-deaminase activity of whole chick-embryo homogenates after injection of adenosine into the air sac*

Incubation time when injected (days)	Adenosine-deaminase activity (units/mg. of protein)		Percentage increase (<i>P</i> 0.05)
	Control	Experimental	
	Dose: 20 mg. of adenosine		
1	0.7	1.4	None
1	1.2	1.8	None
2	6.2	5.5	None
3	10.6	14.5	37
3	7.5	6.4	None
3	7.0	7.1	None
4	7.6	6.8	None
4	9.7	10.9	None
4	14.5	15.4	None
5	13.6	10.8	None
6	9.3	12.7	37
7	8.9	8.3	None
Mean	9.5	9.8	
Dose: 25 mg. of adenosine			
6	4.5	7.6	69
6	7.4	9.7	None
6.5	7.5	9.1	None
7	10.2	21.0	108
7	7.1	9.2	29
7	7.5	14.8	97
7	8.9	9.4	None
8	9.0	8.6	None
Mean	7.7	11.2	

Table 5. *Effect of the injection of 20 mg. of adenosine on the adenosine deaminase of yolk sacs of chick embryos at different stages of development*

Incubation time when injected (days)	Adenosine-deaminase activity (units/mg. of protein)		Percentage decrease (P 0.05)
	Control	Experimental	
1	4.3	4.6	None
1	2.8	3.4	None
2	13.3	15.1	None
2	5.8	3.6	38
4	5.1	3.3	35
4	14.0	9.7	31
4	13.1	14.8	None
5	7.2	4.4	39
6	16.0	13.3	None
8*	25.4	16.2	36
8*	28.3	32.9	None
Mean	11.4	11.0	

* Dosed with 25 mg. of adenosine.

Although adenosine-deaminase activity does not appear to be localized in the liver, it was thought that the liver might be more likely to show enzyme adaptation than the whole chick embryo. As Gordon & Roder (1953) had obtained a sevenfold increase after seven injections of 10 mg. of adenosine during 7–13 days of incubation, it was thought that one dose of 20–25 mg. of adenosine might promote some increase of adenosine-deaminase activity. However, apart from an increase of 47% in the liver of a 14-day-old embryo receiving 15 mg. of adenosine, no increased activity was produced with either single or multiple injections of adenosine (Table 6). Neither two injections of 15 mg. of adenosine at 24 hr. intervals nor five injections of 10 mg. of adenosine at 24 hr. intervals resulted in any increase of activity of the liver adenosine deaminase at 24 hr. after the last injection. As a short-lived adaptation might have occurred during the 24 hr. post-injection period an experiment was carried out on 80 eggs which had been incubated for 7 days. The eggs were injected with 25 mg. of adenosine and the livers removed at 6, 9, 12, 16, 18 and 24 hr. after injection. No significant increase of adenosine-deaminase activity occurred during this period. Chicks hatched from eggs dosed with 25 mg. of adenosine at 6 days of incubation showed no morphological abnormalities.

A preliminary report by Gordon (1952) on the effect of injecting adenosine into the chick egg suggested that liver-adenosine-deaminase activity could only be increased in embryos which were at least 20 days old. In view of the hypothesis (Burnet, 1956) that enzyme adaptation may be a preliminary step to antibody production and as it is known that the chick is not capable of producing antibodies until after hatching (Tyler, 1955), the reaction of the liver adenosine deaminase of chicks and fowls to injections of adenosine was briefly examined. In chicks and fowls such an experiment

is severely limited not by the toxicity of adenosine but by the volume of solution of the poorly soluble adenosine that can safely be injected intraperitoneally. In this work the most that was injected daily into 20-week-old cockerels was 300 mg. of adenosine in 18 ml. of 0.9% NaCl and four successive doses produced no ill-effects on the birds. A single dose of 300 mg. of adenosine injected into a 19-week-old cockerel produced a 37% increase of liver-adenosine-deaminase activity after 24 hr.; four successive injections of 300 mg. of adenosine into a 20-week-old cockerel at 24 hr. intervals produced a 57% increase in the adenosine deaminase of the liver, but not of the spleen (15.7 activity units/mg. of protein), at 24 hr. after the last injection (Table 6). However, the mean values for all the control and experimental chick embryo and chick livers were not significantly different.

The amount of ammonia evolved from homogenates of untreated embryos without substrate in all these determinations showed no significant variation during development. Both whole chick embryo and liver homogenates evolved 0.18 ± 0.03 (50 results) $\mu\text{g. of ammonia/30 min./mg. of protein}$, and yolk-sac homogenates evolved 0.09 ± 0.02 (10 results) $\mu\text{g. of ammonia/30 min./mg. of protein}$.

DISCUSSION

The distribution of adenosine deaminase in the various tissues of the chick has not been extensively studied. Whereas Conway & Cooke (1939) found that there was about ten times more adenosine-deaminase activity in rabbit spleen than in the liver, in this work it was found that in a 19-week-old cockerel the adenosine-deaminase activity/mg. of protein in the liver does not significantly differ from that in the spleen. Gustafson & Hasselberg (1951) have found that no variation of adenosine-deaminase activity/mg. of nitrogen occurred

Table 6. *Effect of injections of adenosine on the adenosine-deaminase activity of the livers of chick embryos, chick and fowls*

	Age when first injected (days)	Dose of adenosine (mg.)	Adenosine-deaminase activity (units/mg. of protein)		Percentage increase (<i>P</i> 0.05)
			Control	Experimental	
Embryos	6	2 × 15	9.1	9.8	None
	6	5 × 10	17.2	10.7	None
	7	15	7.2	7.7	None
	7	25	6.1	6.3	None
	7	25	7.8	7.2	None
	9	15	6.2	7.5	None
	14	15	6.3	9.3	47
Chicks and fowls	8	3 × 25	9.0	8.6	None
	133	300	7.3	10.1	37
	140	4 × 300	10.8	16.8	57
Mean			8.7	9.4	

during the early development of *Paracentrotus lividus*. However, adenosine deaminase has been found to increase in mouse liver after injection of xanthine (Feigelson, Feigelson & Wood, 1954).

Contrary to earlier beliefs that embryonic differentiation was accompanied by the synthesis of many enzymes, it appears from recent work that many enzyme activities show only relative changes of activity during development (e.g. Shen, 1955; Moog, 1946, 1947, 1950; Solomon, 1957, 1958, 1959). It is unlikely that a tissue having high enzyme activity will respond to the stimulus of a large amount of substrate as there will always be sufficient enzyme to deal with even large amounts of substrate. This is one reason for testing enzymes known to have only low activities (e.g. adenosine deaminase and glutaminase). Even then the study of any such adaptation is severely limited, as adaptation may occur only at substrate doses very near the 50% lethal level, as was found for arginase in the chick embryo (Roeder, 1957). Gordon (1956) also reports that a major difficulty of studying enzyme adaptation is the high death rate of chick embryos injected with the various substrates. In this work adenosine has also been found to be toxic to the chick embryo and this may be partly due to its specific action *in vitro* of inhibiting cells from entering prophase (Hughes, 1952); no morphological or growth-inhibiting effects of adenosine have been noticed in these experiments.

No substrate-induced increase of adenosine deaminase at any stage of development of the chick embryo has been found in this work. This is contrary to the results obtained by Gordon & Roder (1953) after the injection of 25 mg. of adenosine in the whole chick embryo at 7 days of development and in the embryonic liver after seven successive doses of 10 mg. of adenosine. Moreover, it has been shown in this work that the activity of adenosine deaminase/mg. of protein does not show any significant increase in untreated whole chick embryos (after 2 days of incubation), yolk sacs or livers throughout development. This, again, is in disagreement with the results of Gordon & Roder (1953) on whole chick-embryo homogenates over a narrower range of development. These authors found that adenosine-deaminase activity/mg. of protein increased from about 2 units at 7 days of incubation to about 20 units at 14 days of incubation in untreated whole embryos. The range of activities obtained in the present work during this period of incubation was 4.5–14.9 units, but there was no upward trend in the values as time of incubation increased. From the results of the present experiments adenosine deaminase can be considered to be a constitutive enzyme throughout the development of the chick embryo.

SUMMARY

1. Adenosine-deaminase activity/mg. of protein does not vary significantly in homogenates of the whole chick embryo after 2 days of incubation or of its yolk sac from 2 to 14 days of incubation or in the liver from 7 days of incubation to 34 weeks after hatching.

2. Adenosine was injected into eggs at from 1 to 8 days of incubation but no significant increase of adenosine-deaminase activity in homogenates of the whole chick embryo or yolk sac was observed at 24 hr. after each injection.

3. Five successive doses of 10 mg. of adenosine injected at 24 hr. intervals into 6-day-old embryos produced no increased adenosine-deaminase activity in the livers of the embryos removed 24 hr. after the last dose.

4. Liver adenosine deaminase was not significantly increased by single or multiple injections of adenosine into embryos during 6–8 days of incubation or by similar injections into chicks or fowls.

5. Adenosine-deaminase activity was chiefly localized in the supernatant fraction of a liver homogenate of a 34-week-old cockerel and its optimum activity in this fraction was at pH 7.0.

6. Adenosine-deaminase activity/mg. of protein was similar in embryonic and cockerel liver, whole chick embryos and yolk sacs.

7. It is concluded from these results that adenosine deaminase is a constitutive and not an adaptive enzyme throughout the development of the chick.

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Aromatic N-Deacylation by Chick-Kidney Mitochondria

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During an investigation of the metabolism of some *para*-substituted anilines it became desirable to convert the excreted *N*-acetyl compounds into their arylamines by enzymic rather than by acid hydrolysis, so reducing the risk of altering other parts of the metabolite molecules. Kynurenine formamidase of mammalian liver (Mehler & Knox, 1950) and of *Neurospora* (Jakoby, 1954), the only enzymes of this type described, appeared not to be suitable because of their high specificity for *o*-formamido compounds. There was, however, evidence that an enzyme more appropriate to our requirements was to be found in chick kidney. Shaffer & Bieter (1950) reported that after giving sulphanilamides to chicks much less of the *N*⁴-acetyl conjugates could be found in kidney than in other tissues or blood. They also found that administered *N*⁴-acetylsulphanilamides were excreted in the urine largely as the free bases; this was in contrast with mammalian species. Finally, these workers showed that chick-kidney homogenates deacetylated acetylsulphanilamides at a rapid rate.

Some of the properties of the chick-kidney enzyme have been explored and are now reported. In spite of the superficial similarity of the reaction it catalyses, the enzyme is quite distinct from kynurenine formamidase.

EXPERIMENTAL

Materials

Substrates. A few of the compounds tested as substrates were of commercial origin. In most cases the arylamines, obtained commercially, were acylated with the appropriate acid anhydride or acid chloride. Formylation was carried out with acetic formic anhydride (Dalglish, 1952). In every case the compound was recrystallized from a suitable solvent (e.g. aqueous ethanol) until all detectable arylamine had been removed. The *p*-acetamido derivatives of 3-phenoxypropionic acid, 4-phenoxybutyric acid and 5-phenoxyvaleric acid were kindly provided by Dr A. G. Caldwell. Compounds in the benzamido series were generously prepared for the present purpose by Dr J. H. Gorvin.

Buffers. Buffered solutions at a concentration of 0.1 or 0.2 M were prepared in glass-distilled water. 2-Amino-2-hydroxymethylpropane-1:3-diol (tris) and 2-amino-2-methylpropane-1:3-diol (ammediol) were obtained from L. Light and Co. Ltd. The pH values of all solutions were measured at the concentration at which they were used, with a glass electrode assembly (W. G. Pye and Co. Ltd.) and temperature corrections were applied where necessary.

Other reagents. Most reagents were of A.R. grade and were used as such; others were purified, when necessary, by some standard procedure appropriate to the particular compound. *p*-Chloromercuribenzoic acid was a gift from Dr L. A. Stocken; 2:3-dimercaptopropanol was bought from Boots Pure Drug Co. Ltd. and was not purified. Tween 20 (Honeywill-Atlas Ltd.) and Triton X-100 (Rohm