4. Possible reasons for the absence of conjugation in other studies of steroid metabolism *in vitro* are discussed.

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The Effects of Anoxia on Metabolism in the Adult Housefly, *Musca domestica*

By G. M. PRICE

Biochemistry Department, Agricultural Research Council, Pest Infestation Laboratory, London Road, Slough, Bucks.

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In contrast with most mammals many insects are able to remain alive under completely anoxic conditions for several hours and in some cases even for days (Brand, 1946). Changes in the concentrations of the intermediates of basal metabolism under conditions of anoxia are therefore of considerable interest, for in the insect these changes are, presumably, reversible and of such a nature that they do not have any serious deleterious effect on the insect's metabolism. The effect of anoxia on the concentrations of glycolytic end products in insects has been investigated by various authors. Gilmour (1941) showed that the amount of lactate accumulating in larvae of *Tenebrio molitor* under anoxic conditions was not sufficient to account for the glycogen and sugar utilized. Barron & Tahmisian (1948) and Humphrey (1949) found that

there was only a small accumulation of lactate, and that pyruvate did not accumulate at all in their preparations of cockroach-leg muscle unless glycogen was added. Chefurka (1958) observed that the sum of pyruvate, lactate and α -glycerophosphate accumulating in cockroach-leg muscle under anoxic conditions was less than the amount of hexose diphosphate utilized. These observations suggested that some end product, in addition to those mentioned above, was accumulating in these insect preparations. It has been suggested (Price, 1961b) that this end product might be α -alanine, and in the present work evidence for this suggestion is examined and the effects of anoxia on various amino acids, on citric acid-cycle intermediates and on the incorporation of amino acids into protein are investigated.

METHODS

Chemicals. The L- or DL-forms of amino acids were purchased from L. Light and Co. Ltd., Colnbrook, Bucks. Each amino acid gave only one ninhydrin-positive spot after two-dimensional chromatography (see below) and was therefore used without further purification. Anhydrous sodium $[2\text{-}^{14}\text{C}]$ acetate, with a specific radioactivity of $5\text{--}15\text{ }\mu\text{Ci}/\mu\text{mole}$, was purchased from The Radiochemical Centre, Amersham, Bucks.

Breeding of houseflies. Adult houseflies (*Musca domestica*) were kept at 27° and were fed on glucose, milk and water. Only females, 4–8 days old, were used in the experiments.

Two-dimensional chromatography. Descending two-dimensional chromatography was carried out in a conventional manner on sheets of Whatman no. 1 paper, with butan-1-ol-acetic acid-water (5:1:4, by vol.) (Phillips, 1949) in the first direction (solvent 1) and water-saturated phenol-aq. 0.1% NH_3 (Conden, Gordon & Martin, 1944) in the second direction (solvent 2). The chromatograms were not equilibrated with the aqueous phase before development in the first solvent. On the other hand with the second solvent the chromatograms were equilibrated overnight with the aqueous phase before being developed. These procedures considerably reduced 'tailing' of the amino acids in the two solvents.

Location and identification of amino acids. Amino acids were located by dipping the chromatogram in 0.5% (w/v) ninhydrin in acetone-1% (v/v) acetic acid, and heating the chromatogram at 90° for 30 min. They were identified by comparing chromatograms of the extracts with those of known amino acids.

Determination of amino acids. After treatment with ninhydrin, as described above, the amino acid spot was cut into small pieces and agitated with a glass rod in 4 ml. of aq. 60% (v/v) ethanol. The solution was centrifuged at $2100g$ for 10 min. and the absorption of the supernatant was measured at $570\text{ m}\mu$ against a blank prepared from paper treated in an identical manner. The above method is similar to that described by Roland & Gross (1954). All estimations were made in triplicate, and by the above method standard amounts of amino acids (0–20 $\mu\text{g.}$) were estimated with an error of $\pm 5\%$.

Determination of proline. Proline was determined by the method of Troll & Lindsley (1955), with an error of $\pm 5\%$.

Location of organic acids. Organic acids can be located on chromatograms by dipping the latter in ethanolic bromocresol green (see Block, Durrum & Zweig, 1958). In the present work 0.2% (w/v) bromocresol green in acetone-water (5:1, v/v) was used.

Location of ^{14}C -labelled compounds and measurement of ^{14}C -activity. ^{14}C -Labelled compounds were located on two-dimensional chromatograms by radioautography. The chromatogram was then cut into strips so that each strip contained one or more of the labelled compounds and the ^{14}C activity was measured with a 4π scanner (Winteringham, 1958). When standard amounts of $[2\text{-}^{14}\text{C}]$ acetate were measured in this manner the coefficient of variation was 5.4%.

Identification of ^{14}C -labelled compounds other than amino acids. Having located a ^{14}C -labelled compound by radioautography its position on the chromatogram was outlined in pencil. The criterion of identification was exact coincidence in position and shape of the ^{14}C -labelled compound

with an added marker compound in solvents 1 and 2, and in solvent 3 [ethylCellosolve-aq. NH_3 (sp.gr. 0.88)-water (16:1:3, by vol.) (Cheftel, Munier & Macheboeuf, 1952)].

Treatment of flies. Flies were lightly anaesthetized with cyclopropane and then injected with $4\text{ }\mu\text{Ci}$ of $[2\text{-}^{14}\text{C}]$ acetate as described by Price (1961a). The flies, in five groups of ten, were put into small glass cylinders, the open ends of which were covered with removable wire-mesh disks, and they were left in air for 60 min. Group 1 was then killed in liquid nitrogen [aerobic flies (1)]. Groups 2 and 3 were left in air for a further 60 min. and 120 min. respectively before being killed [aerobic flies (2) and (3)]. Groups 4 and 5 were put into oxygen-free nitrogen for 60 min. and 120 min. respectively before being killed [anoxic flies (4) and (5)].

Extraction of flies. The flies of each group were ground in liquid nitrogen to a powder which was homogenized in 1 ml. of aq. 95% (v/v) ethanol at 0° , and the homogenate was centrifuged at $2100g$ for 10 min. The residue was homogenized in 1 ml. of aq. 50% (v/v) ethanol at room temperature and centrifuged as before. The combined supernatants were centrifuged at $22\text{ }000g$ for 30 min. and the volume of the supernatant from this centrifuging was made up to 2 ml. with ethanol. A sample (100 $\mu\text{l.}$) of this extract was chromatographed as described above.

Extraction and determination of ^{14}C -labelled protein amino acids. The residue obtained, after ^{14}C -labelled flies had been extracted twice with ethanol (see above), was homogenized twice with 1 ml. of N-HCl, twice with 1 ml. of ethanol-ether (3:1, v/v), twice with 1 ml. of acetone and once with 1 ml. of methanol-chloroform (1:1, v/v). Each homogenate was centrifuged at $2100g$ for 10 min., the supernatant was discarded and the final residue was dried in a desiccator over CaCl_2 . It was hydrolysed in 6 ml. of 6N-HCl at 108° for 16 hr. The hydrolysate was filtered and the flask washed twice with 2 ml. of aq. 95% (v/v) ethanol, the washings also being filtered. In order to remove the HCl the combined filtrates were concentrated by evaporation (three times) to less than 0.2 ml. Aqueous 95% (v/v) ethanol (5 ml.) was added after the first and second evaporations. After the third evaporation the flask was rinsed with aq. 95% (v/v) ethanol to give an extract of a total volume of 2 ml. Samples (20 $\mu\text{l.}$ or 50 $\mu\text{l.}$) of this extract were chromatographed two-dimensionally. The amino acids were located and identified, and their chemical amounts and the ^{14}C activity measured as described above.

Extraction and determination of pyruvic acid. Flies, in groups of twenty, were killed in liquid nitrogen and ground to a powder, which was homogenized at 0° in 3 ml. of freshly prepared 2% (w/v) tungstic acid (Cavallini & Frontali, 1954). The homogenate was left at room temperature for 10 min. after which it was centrifuged at $2100g$ for 15 min. The supernatant was filtered through Whatman no. 50 paper and the residue in the tube homogenized with 1 ml. of 2% (w/v) tungstic acid at room temperature. This homogenate was centrifuged and the supernatant filtered as described above. The combined filtrates were made up to 5 ml. with water and 1 ml. of 0.2% (w/v) 2,4-dinitrophenylhydrazine in 2N-HCl was added and the subsequent estimation of the 2,4-dinitrophenylhydrazone of pyruvate was carried out as described by Tauber (1955), except that diethyl ether was used in place of ethyl acetate and the final measurements were made at $440\text{ m}\mu$ on a Unicam spectrophotometer.

RESULTS

Identification of ^{14}C -labelled intermediates. A tracing of a radioautograph obtained from a two-dimensional chromatogram of the ethanol extract of anoxic flies (5) is shown in Fig. 1. The ^{14}C -labelled amino acids present in the extract were the same as those reported by Price (1961*a*). One ninhydrin-positive ^{14}C -labelled compound coincided with added glutathione in solvents 1 and 2 (spot 1, Fig. 1) and disappeared when the extract was hydrolysed with 6*N*-hydrochloric acid. The ethanol extract also contained a number of ^{14}C -labelled compounds which were not ninhydrin-positive, and their distribution on the chromatogram was similar to that of ^{14}C -labelled compounds identified as citric acid-cycle intermediates by Katz & Chaikoff (1955). Four of these compounds coincided with fumarate, lactate, malate and succinate respectively in solvents 1, 2 and 3. Citrate, isocitrate, *cis*-aconitate, α -oxoglutarate, pyruvate, oxaloacetate, and the glycolytic intermediates dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, α -glycerophosphate, 3-phosphoglycerate and phosphoenolpyruvate, in 100 μg . amounts, were also chromatographed in solvents 1 and 2, but they did not coincide with any of the ^{14}C -labelled compounds. α -Oxoglutarate streaked badly in these solvents and a large per-

Fig. 1. Tracing of a radioautograph obtained from a two-dimensional chromatogram of the ethanol extract of anoxic flies (5) (see Table 1). Details of the chromatography are given in the text. Spots: 1, glutathione; 2, aspartate; 3, glutamate; 4, α -alanine; 5, glutamine; 6, proline; 7, γ -aminobutyrate; 8, unidentified compound; 9, malate; 10, unidentified compound; 11, succinate; 12, fumarate; 13, lactate; 14 and 15, unidentified compounds.

centage of the pyruvate applied to the chromatograms volatilized off during their development. Because of their poor behaviour in these solvents and because they are present in only small amounts in the fly it was not possible to tell whether or not these two keto acids were labelled. The dinitrophenylhydrazones of the keto acids present in five aerobic flies (1) were separated by paper electrophoresis on Whatman no. 1 paper. Only two derivatives were detectable and during electrophoresis they behaved in a manner identical with that of the authentic derivatives of pyruvate and α -oxoglutarate respectively. Each derivative also coincided exactly with a peak of ^{14}C activity.

Effect of anoxia on free amino acids. The chemical concentrations and the specific activities of five amino acids in the aerobic and anoxic flies are shown in Table 1. In the changes reported below, aerobic flies (1) are taken as the zero-time control group for aerobic flies (2) and (3) and for anoxic flies (4) and (5). In the aerobic flies there was little change in the chemical amounts of aspartate, glutamate and glutamine, whereas the concentrations of α -alanine and of proline fell by 1.6 and 1.3 $\mu\text{moles/g}$. wet wt. of fly respectively. A similar fall in the concentration of α -alanine has been observed in houseflies that had been starved for 3 hr. (Dr K. A. Lord, personal communication). In the anoxic flies, the concentration of α -alanine rose from 4.5 to 15.9 $\mu\text{moles/g}$. wet wt. of fly, whereas that of glutamine fell from 3.5 to 1.6 $\mu\text{moles/g}$. wet wt. of fly, and there was an almost complete depletion of aspartate. In the aerobic flies the specific activity of each amino acid apart from proline fell, as observed by Price (1961*a*). In the anoxic flies there was no change in the specific activity of α -alanine but that of glutamate and of glutamine fell. The ^{14}C activity of aspartate in these flies fell to a very low level though that of γ -aminobutyrate increased. The results show that the specific activity of α -alanine is less than that of proline in aerobic flies (1) (Table 1), whereas in previous work (Price, 1961*a*) the specific activity of α -alanine was greater than that of proline in flies at a corresponding stage of 'labelling'. No explanation can be offered at present for these differing results. The specific activities of glutamate and glutamine, however, agree reasonably well with those previously obtained.

Effect of anoxia on citric acid-cycle intermediates. The ^{14}C activity of these intermediates and of the unidentified compound 8 fell in the aerobic flies (Table 2), this being a similar result to that obtained with the ^{14}C -labelled free amino acids. In the anoxic flies the ^{14}C activity of fumarate fell, though that of malate rose, during the first hour of anoxia, and then returned to its original level after 2 hr. of anoxia. There was a fivefold increase in the ^{14}C

activity of succinate during 2 hr. of anoxia and there was a considerable amount of ^{14}C activity in lactate and in the unidentified compounds 10, 14 and 15. The fact that ^{14}C -labelled lactate was not detectable in the aerobic flies, but was detectable in the anoxic flies, suggests that there was a chemical accumulation of lactate in the anoxic flies.

Pyruvate was measured in flies after various periods of anoxia and the results are shown in Fig. 2. Since large numbers of flies were required for these measurements they were not injected with $[2\text{-}^{14}\text{C}]\text{acetate}$. The concentration of pyruvate

rose rapidly during the first 30 min. of anoxia from 0.3 to 1.9 $\mu\text{moles/g. wet wt. of fly}$, but returned to its original level after 2 hr. of anoxia. Pyruvate was also measured in two groups of ^{14}C -labelled flies. One group corresponded in time to aerobic flies (1) and the other group was one in which the flies were anoxic for 30 min. The ^{14}C activity of pyruvate rose from 0.1 to 0.2 $\mu\text{C/g. wet wt. of fly}$ during 30 min. of anoxia and the chemical concentration rose from 0.2 to 2.0 $\mu\text{moles/g. wet wt. of fly}$. As a result of these changes the specific activity of pyruvate fell from 0.5 to 0.1 $\mu\text{C}/\mu\text{mole}$ during 30 min. of anoxia.

Table 1. *Chemical concentrations and specific activities of ^{14}C -labelled free amino acids in aerobic flies and in anoxic flies*

A sample (100 $\mu\text{l.}$) of extract, the equivalent of half a fly, was chromatographed, and the chemical concentrations and the ^{14}C activity of the amino acids were measured, as described in the text. The ^{14}C activity of the amino acids in 100 $\mu\text{l.}$ of extract was over the range 500–15 000 counts/min. Under the scanning conditions employed 198 600 counts/min. were equivalent to 1 μC of ^{14}C activity. Aerobic flies were left in air for (1) 60 min., (2) 120 min. or (3) 180 min. after injection with $[2\text{-}^{14}\text{C}]\text{acetate}$. Anoxic flies were left in air for 60 min. after injection with $[2\text{-}^{14}\text{C}]\text{acetate}$ and were then put into oxygen-free nitrogen for (4) 60 min. or (5) 120 min. —, Values too low to give reliable measurements.

	Chemical concentration ($\mu\text{moles/g. wet wt. of fly}$)				
	Aerobic flies			Anoxic flies	
	(1)	(2)	(3)	(4)	(5)
α -Alanine	4.5	2.9	2.9	14.2	15.9
Aspartate	2.8	2.8	2.8	—	—
Glutamate	5.4	5.4	5.7	4.3	4.0
Glutamine	3.5	4.0	3.9	2.2	1.6
Proline	6.2	5.8	4.9	5.1	4.9
	Specific activity ($\mu\text{C}/\mu\text{mole}$)				
	Aerobic flies			Anoxic flies	
	(1)	(2)	(3)	(4)	(5)
α -Alanine	0.3	0.2	0.2	0.3	0.3
Aspartate	0.9	0.6	0.5	—	—
Glutamate	2.5	1.2	0.9	2.0	1.4
Glutamine	1.1	0.9	0.8	0.6	0.4
Proline	1.3	1.2	1.3	1.1	1.0

Table 2. *^{14}C activity of ^{14}C -labelled compounds in aerobic flies and in anoxic flies*

A sample (100 $\mu\text{l.}$) of extract was chromatographed and the ^{14}C activity was measured, as described in the text. The ^{14}C activity of the compounds was over the range 600–22 000 counts/min. Aerobic flies (1), (2) and (3), and anoxic flies (4) and (5), are defined in Table 1. The unidentified compounds are numbered according to the spot nos. in Fig. 1.

^{14}C -Labelled compound	^{14}C activity ($\mu\text{C/g. wet wt. of fly}$)				
	Aerobic flies			Anoxic flies	
	(1)	(2)	(3)	(4)	(5)
Fumarate	0.5	0.2	0.1	0.3	0.3
Lactate	0	0	0	1.4	2.0
Malate	1.6	0.9	0.5	2.5	1.6
Succinate	1.9	0.8	0.4	12.3	10.0
Unidentified compound 8	6.6	2.6	1.6	3.3	0.5
Unidentified compound 10	0	0	0	5.3	5.1
Unidentified compound 14	0	0	0	1.7	2.1
Unidentified compound 15	0	0	0	1.1	1.1

Table 3. *Specific activities of ^{14}C -labelled protein amino acids in aerobic flies and in anoxic flies*

A sample (50 $\mu\text{l.}$) of extract was chromatographed and the chemical concentrations and ^{14}C activities were measured as described in the text. The ^{14}C activity of the amino acids in 50 $\mu\text{l.}$ of extract was over the range 150–1000 counts/min. Aerobic flies (1) and (3) and anoxic flies (5) are defined in Table 1.

Amino acid	$10^2 \times \text{Specific activity } (\mu\text{C}/\mu\text{mole})$		
	Aerobic flies		Anoxic flies
	(1)	(3)	(5)
α -Alanine	0.5	1.3	0.5
Aspartate	1.3	2.4	1.0
Glutamate	3.8	5.8	1.8
Proline	0.9	2.6	0.4

Effects of anoxia on the incorporation of amino acids into protein. The specific activities of the ^{14}C -labelled protein amino acids found after protein hydrolysis are shown in Table 3. In the aerobic flies the specific activities of the protein amino acids increased, but in the anoxic flies the specific activities either decreased or remained the same. The results show that in the aerobic flies free ^{14}C -labelled amino acids were incorporated into protein whereas in the anoxic flies there was no incorporation.

DISCUSSION

^{14}C -labelled α -alanine, aspartate, glutamate, glutamine, γ -aminobutyrate, glutathione, fumarate, malate, α -oxoglutarate, pyruvate and succinate were found in houseflies after they had been injected with $[2\text{-}^{14}\text{C}]\text{acetate}$. These compounds were also found in anoxic flies but in addition these flies contained ^{14}C -labelled lactate and at least three unidentified ^{14}C -labelled compounds.

^{14}C -labelled free amino acids were incorporated into protein in the aerobic flies, whereas in the anoxic flies there was no incorporation. The inhibition by anaerobiosis of the incorporation of ^{14}C -labelled amino acids into protein has also been reported by Frantz, Zamecnik, Reese & Stephenson (1948) for rat-liver slices and by Borsook, Deasy, Haagen-Smit, Keighley & Lowy (1950) with bone-marrow cells. This inhibition is not surprising for it is well-established, as pointed out in various reviews (Hoagland, 1958; Halvorson, 1960), that the incorporation of amino acids into protein is an energy-requiring process and, since ATP is severely depleted in anoxic flies (Winteringham, 1960; Heslop, Price & Ray, 1962), there may be only very little, if any, of this nucleotide available for protein synthesis.

Pyruvate accumulated rapidly during the first 30 min. of anoxia (Fig. 2); however, after this time its concentration fell, indicating that it was being

Fig. 2. Concentration of pyruvate in the housefly over 2 hr. of anoxia.

further metabolized. The increase of ^{14}C activity in lactate in the anoxic flies indicates that some of the pyruvate was further metabolized to lactate. Barron & Tahmisian (1948), working with teased fibres of cockroach-leg muscle, observed that the concentration of pyruvate after 1 hr. of anoxia was the same as that in the zero-time control, and that there was only a slight increase in lactate. Similarly, Humphrey (1949) showed that, unless glycogen was added, there was only a small increase in pyruvate and lactate in homogenates of cockroach-leg muscle incubated anaerobically. The concentrations of pyruvate cited by these authors are for periods of not less than 1 hr. of anoxia and therefore it is not possible to tell whether there was a rise and fall of this acid in their preparations during the first hour of anoxia.

Price (1961b) has suggested that α -alanine could be a glycolytic end product and that its formation from pyruvate might account for the finding (Chefurka, 1958) that the sum of α -glycerophosphate, lactate and pyruvate accumulating in cockroach-leg muscle under anaerobic conditions was less than the amount of hexose diphosphate utilized. Since the chromatographic results indicate that after injection with $[2\text{-}^{14}\text{C}]\text{acetate}$ the glycolytic intermediates in the fly are not labelled, then during anoxia if the pyruvate and α -alanine which accumulate were derived solely from these intermediates one would expect the specific activities of both these compounds to fall in the anoxic flies. That of pyruvate does fall during 30 min. of anoxia, but only fivefold and not tenfold as one might expect from its chemical accumulation. This result indicates that, although some pyruvate is

Scheme 1. Suggested pathways of reactions taking place in anoxic flies. The compounds in which there is a chemical increase or an increase in ^{14}C activity or both are boxed in continuous lines. Those in which there is a decrease are boxed in broken lines.

probably derived from the unlabelled glycolytic intermediates, some must also be derived from a labelled source. The specific activity of α -alanine does not fall at all but remains constant during 2 hr. of anoxia (Table 1), which indicates that this amino acid cannot be derived solely from pyruvate but that it must also be derived from some other labelled precursor, e.g. aspartate, from which it can be obtained by β -decarboxylation. That decarboxylation can take place in the anoxic flies is indicated by the increase in these flies of γ -aminobutyrate, the α -decarboxylation product of glutamate. It is not possible to tell from the results how much of the α -alanine accumulating is of glycolytic origin. Depending on the amount it could partially or wholly account for the differences observed by Chefurka (1958).

The fall in the chemical concentration and in the specific activity of glutamine suggests that this amino acid is being 'turned over' in the anoxic flies, probably resulting in the production of glutamate and ammonia. Finally, it is suggested that the changes observed in the anoxic flies are due to the reactions outlined in Scheme 1.

SUMMARY

1. Houseflies were injected with $[2-^{14}\text{C}]$ acetate and were left in air for 1 hr. Some were then killed, some were left in air for a further 1 hr. or 2 hr., and others were put into oxygen-free nitrogen for 1 hr. or 2 hr.

2. In the aerobic flies ^{14}C -labelled α -alanine, aspartate, glutamate, glutamine, proline, γ -amino-

butyrate, fumarate, malate, α -oxoglutarate, pyruvate, succinate and glutathione were present.

3. In the anoxic flies all the above intermediates were present as well as ^{14}C -labelled lactate and three other unidentified ^{14}C -labelled intermediates.

4. In the anoxic flies there was an increase in the concentration of α -alanine, but there was a fall in the concentrations of aspartate and of glutamine. The concentration of pyruvate rose during the first 30 min. of anoxia but later returned to its original value. There was a rise in the ^{14}C activity of lactate, of succinate and of γ -aminobutyrate.

5. In the aerobic flies the specific activity of the protein amino acids increased whereas in the anoxic flies their specific activity either decreased or remained the same.

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Detection and Resolution of Gangliosides in Lipid Extracts by Thin-Layer Chromatography

By J. R. WHERRETT* AND J. N. CUMINGS

Department of Chemical Pathology, Institute of Neurology, The National Hospital, Queen Square, London, W.C. 1

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Ganglioside was the name given by Klenk (1942) to a lipid substance located predominantly in the grey matter of the brain which, on hydrolysis, released cerebroside and other carbohydrate compounds. Among these were identified hexose and neuraminic acid (Klenk, 1942) as well as hexosamine (Blix, Svennerholm & Werner, 1950, 1952; Klenk, 1951). Similar lipid-soluble, neuraminic acid-containing substances extracted and purified by a variety of methods have been described (Folch, Arsove & Meath, 1951a; Svennerholm, 1956a; Bogoch, 1958; Rosenberg & Chargaff, 1958; Folch & Lees, 1959; van Heyningen & Miller, 1961; Payne & Platt, 1961; Svennerholm & Raal, 1961; Trams & Lauter, 1962), which differ with respect to percentage composition and presence of non-glycolipid components, but the relationship between these various preparations is not entirely clear. Evidence that these preparations may contain different combinations of aminoglycolipids (both neuraminic acid and non-neuraminic acid-containing), glycosaminoglycans and glycopeptides has been presented by Svennerholm (1956b) and Bogoch, Belval & Winer (1961). It is now usual, however, to reserve the term ganglioside for glycolipids that contain neuraminic acid, irrespective of the tissue of origin. Ganglioside preparations are extracted from tissues only with lipid solvents

of a mild polar nature and are usually water-soluble, exhibiting in aqueous solution the physical properties of a single large molecule (Folch *et al.* 1951a; Chatagnon & Chatagnon, 1953; Bogoch, 1958; Rosenberg & Chargaff, 1958). Sedimentation behaviour in dimethylformamide as opposed to water (Klenk & Gielen, 1960) and titration data (Klenk, 1942) are consistent with a much smaller molecular weight and the heterogeneity of ganglioside revealed by countercurrent distribution (Meltzer, 1958) and chromatography (Svennerholm, 1956a; Kuhn, 1958, 1959; Kuhn *et al.* 1960; Kuhn, Weigandt & Egge, 1961; Weicker, Dain, Schmidt & Thannhauser, 1960; van Heyningen & Miller, 1961; Klenk & Gielen, 1961a, b, c; Payne & Platt, 1961; Dain, Weicker, Schmidt & Thannhauser, 1962a; Dain, Willis, Sweet & Schmidt, 1962b) would suggest that the physical behaviour in water results from the aggregation of smaller molecular species to form micelles.

The fractionation of ganglioside by chromatography on thin layers of silica gel appears to be highly effective. The high resolving power of the method makes it possible to separate the components of a lipid class such as the gangliosides even in the presence of other tissue lipids (Jatzkewitz, 1961). A particular advantage of thin-layer over paper chromatography is that detection methods of high specificity and sensitivity for neuraminic acid may readily be used. In this work the technique

* Present address: Department of Medicine, Toronto General Hospital, 100 College Street, Toronto 2, Canada.