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The Metabolism and Glutamic Acid Content of Rat Brain in Relation to Thiopentone Anaesthesia

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It has recently been shown that the free glutamic acid content of the rat brain falls during insulin hypoglycaemia (Dawson, 1950). This observation suggests that in the physiological emergency produced by a shortage of glucose the brain oxidizes glutamic acid to gain high-energy phosphate bonds for metabolic activity. It is well known that during barbiturate anaesthesia the metabolic activity of the brain, as measured by its consumption of oxygen, slows down in parallel with the reduction of physiological activity (Etsten & Himwich, 1946; Himwich, Homburger, Maresca & Himwich, 1947). It has been suggested from the results of *in vitro* studies that this depressed metabolism is a consequence of inhibition of brain oxidations by the narcotic (Quastel & Wheatley, 1932). The present investigation was

undertaken to determine whether the reduced metabolism of the brain during anaesthesia was associated with a change in the level of the free glutamic acid in the brain. It was found that in the rat during anaesthesia produced by sodium 5-ethyl-5-(1'-methylbutyl)-2-thiobarbiturate (thiopentone sodium) the free glutamic acid content of the brain was reduced by about 30%. Simultaneous observations also showed that thiopentone anaesthesia reduced the concentration of ammonia found in the brain and slightly increased the glutamine content. Observations were also made on the inhibition by thiopentone of the respiration of isolated rat-brain tissue, and the effect of the drug on some enzyme systems in brain tissue capable of producing free ammonia.

METHODS

Young male rats of the Wistar albino strain were used throughout. For the determination of free glutamic acid and glutamine in whole brain, rats weighing between 40 and 80 g. were killed by decapitation and their heads transferred to liquid oxygen within a few seconds. The subsequent procedure has been described previously (Dawson, 1950).

Glutamic acid and glutamine determinations. These substances were estimated by the microbiological method of Krebs (1948) with certain modifications (Dawson, 1950). With some cultures of *Clostridium welchii* S.R.12 it was noticed that brain extracts gave a very slow evolution of CO_2 after the complete decarboxylation of glutamic acid and glutamine. This slow evolution of CO_2 occurred both aerobically and anaerobically. The substrate has not yet been identified, although it was not histidine. The results presented have, where necessary, been corrected for this factor.

Ammonia was estimated by the microdiffusion method of Conway (1947) with the modifications used by Richter & Dawson (1948).

Paper chromatography of the amino-acids in rat-brain extracts. One-dimensional filter-paper chromatograms of the free amino-acids in rat-brain extracts were prepared using phenol as the solvent. The rats were decapitated and the heads immediately immersed in liquid oxygen. The frozen whole brain was removed from the head, crushed, and the powder then shaken with 5 ml. of 12% (w/v) trichloroacetic acid solution contained in a weighed centrifuge tube. After reweighing, the suspension was centrifuged and 2 ml. of the supernatant was extracted twice with 5 ml. of ether. A sample of the ether-extracted solution was spotted on to a filter-paper sheet with an 'Agl'a' micrometer syringe. Brain extracts from anaesthetized and control animals were spotted in pairs and the volume of the sample (approx. 0.1 ml.) adjusted to correspond to a definite weight of brain. Glutamic acid standards were freshly prepared in 10% (w/v) trichloroacetic acid solution and extracted with ether in a similar way to the brain extracts. After chromatography overnight with redistilled phenol, the filter-paper sheets were dried in a current of air at room temperature to avoid loss of the amino-acids (Fowden & Penney, 1950). Thorough drying was found to be essential, as traces of phenol interfered with the subsequent development of the amino-acid spots with ninhydrin. The dried sheets were then sprayed with 0.1% ninhydrin dissolved in *n*-butanol. The resulting chromatogram of the amino-acids showed five distinct spots, the most prominent one containing the free glutamic acid. Although this main spot consisted largely of glutamic acid, an examination of two-dimensional chromatograms showed that it was composite and contained small quantities of other ninhydrin-reacting substances. In two pairs of rats amino-acid determinations on the 'glutamic acid' spots were performed by Mr G. Ansell. The chromatograms were sprayed with 0.1% NaOH to eliminate NH_3 , and the amino-acid N estimated by the colorimetric method of Moore & Stein (1948).

Respiration of minced brain tissue. The direct method of Warburg was used to measure the O_2 uptake of minced brain tissue. Young rats (90–110 g.) were killed and the whole brain rapidly removed, chilled and finely minced with a razor. The minced brain was weighed and suspended in Krebs phosphate-Ringer (200 mg. brain tissue/ml.). Samples of the suspension (1 ml.) were pipetted into Warburg vessels

already containing additional Ringer solution and glucose. Air was used in the gas space to avoid O_2 poisoning (Elliott & Libet, 1942; Mann & Quastel, 1946; Dickens, 1946). Respiration measured over the first hour amounted to approx. 1250 $\mu\text{l./g. wet wt./hr.}$ In some experiments the O_2 consumption was measured in the presence of a substrate after a previous 3 hr. period of autoxidation (Quastel & Wheatley, 1932). In these measurements use was made of a modified Krebs phosphate-Ringer solution containing a high concentration of K; part of the NaCl being replaced by KCl. This high concentration of K tends to stabilize the inhibition of oxidations produced by barbiturates (Jowett & Quastel, 1937).

Thiopentone anaesthesia. A solution containing 10 mg./ml. of sodium 5-ethyl-5-(1'-methylbutyl)-2-thiobarbiturate (Pentothal, Abbott) was injected intraperitoneally. For *in vitro* experiments, freshly prepared sodium thiopentone solution was adjusted to the required pH with HCl and added to the Warburg vessel as a suspension of the free thiobarbituric acid. Purification of the free acid by a one-stage crystallization from ethanol-water had no effect on the melting point (156°) nor any apparent effect on the inhibition of brain respiration.

Glutaminase. The method of Krebs (1935) was used to determine glutaminase activity in a simple extract of whole rat brain. The NH_3 produced by incubation of the extract at 37° with glutamine (British Chemicals and Biologicals Ltd.) at pH 8.5 was measured by the Conway microdiffusion procedure.

Adenylic deaminase. Activity of this enzyme in extracts of rat brain was determined by a modification of the method of Conway & Cooke (1939). Incubation times of less than 1 hr. and a higher temperature (37°) were used. Adenylic acid (Roche) was added to give a final concentration of 0.6%.

RESULTS

The results of microbiological determinations of the ammonia, glutamine and free glutamic acid content of rat brain are presented in Table 1. The free glutamic acid present in a series of brains taken from rats anaesthetized with thiopentone was significantly less than that of a control series (by Student's *t* test, $P < 0.001$). The mean glutamine concentration in the brains of the anaesthetized rats was slightly raised, the increase being on the borderline of statistical significance (by Student's *t* test, $P = 0.05$). The sum of the glutamic acid present as the free acid and that as the amide was, however, significantly less in the anaesthetized rats ($P < 0.001$). This showed that the reduction in the free glutamic acid level could not be solely accounted for by the increased glutamine concentration. The ammonia content of the brain was lower in the rats killed during anaesthesia. It is known that part of this ammonia would be formed after death, since the ammonia content of rat brain increases very rapidly after decapitation (Richter & Dawson, 1948).

When rats are anaesthetized with barbiturates the body temperature gradually falls, unless heat is supplied to the animal. In the anaesthetized rats the rectal temperature fell well below the normal value, and more anaesthetized rats were therefore

Table 1. *Ammonia, glutamine and free glutamic acid content of the brain in normal and anaesthetized rats*

	Dose of thiopentone (mg./kg.)	Duration of anaesthesia (min.)	NH ₃ (mg./100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Free glutamic acid + glutamine (mg. glutamic acid/100 g. tissue)
Controls	—	—	0.8	84	154	238
	—	—	1.1	84	178	262
	—	—	0.7	58	187	245
	—	—	0.6	85	184	269
	—	—	0.6	83	171	254
	—	—	0.5	77	157	234
		Means	0.7	78	172	250
Anaesthetized	60	65	0.4	84	108	192
	60	15	0.4	81	134	215
	50	70	0.3	89	123	212
	50	45	0.3	96	121	217
	60	55	—	81	115	196
	70	55	—	93	93	186
	65	65	0.2	101	112	213
		Means	0.3	89	115	204
Anaesthetized and body temperature maintained	65	55	—	67	173	240
	75	55	—	78	146	224
	65	> 30	0.5	71	148	219
	65	> 30	0.4	83	143	226
	65	75	0.5	87	151	238
		Means	0.5	77	152	229

Table 2. *Ammonia, glutamine and free glutamic acid content of the rat brain after electrical stimulation and variation of the environmental temperature*

	No. of rats	NH ₃ (mg./100 g. tissue)	Glutamine (mg. glutamic acid/100 g. tissue)	Free glutamic acid (mg./100 g. tissue)	Free glutamic acid + glutamine (mg. glutamic acid/100 g. tissue)	Remarks
Controls (mean of 6 rats)	6	0.7 (0.5-1.1)	78 (58-85)	172 (154-187)	250 (234-269)	—
Electrical stimulation (40 V., 1-2 sec., 50 cyc./sec. a.c.)	3	0.8	81	165	246	Killed in convulsions
		1.3	83	164	247	
		0.7	64	183	247	
High environmental temp. (37°; 1.5 hr.)	2	1.0	88	179	267	Rectal temp. 39°
		0.8	73	177	250	
Low environmental temp. (-5°; 1.5 hr.)	2	1.3	83	185	268	Rectal temp. below 34°
		0.6	68	165	233	

examined, in which the body temperature had been maintained. This was conveniently done by keeping them in a biological incubator at 37° during the narcosis. Estimations of the free glutamic acid, glutamine and ammonia present in the brain showed that maintenance of the body temperature during anaesthesia reduced the amount by which the glutamic acid level in the brain fell (Table 1). However, the levels of the free glutamic acid and of glutamic acid + glutamine were still both significantly reduced below the control values. The mean level of glutamine in these rats was normal. It is probable that maintenance of the body temperature during thiopentone anaesthesia reduces the severity of the anaesthesia by aiding detoxication of the drug. A similar result was noted during an investi-

gation of the effect of pentobarbitone anaesthesia on the phosphorus metabolism of the brain (Dawson & Richter, 1950a). Further experiments were made in which young rats were subjected to high (37°) and low (-5°) environmental temperatures for 1.5 hr. before killing. Estimations of the glutamine and free glutamic acid content of the brain showed that these were within the normal range (Table 2).

In three experiments the concentrations of glutamine and free glutamic acid in the brain were measured in rats which had been killed during convulsions induced by electrical stimulation of the brain (Richter & Dawson, 1948). This increased activity of the brain over a short period of time had no effect on the level of glutamine or free glutamic acid in the organ. Haber & Saidel (1948) have reported

a lowering of the free glutamic acid content of the brain in rats killed during strychnine convulsions.

Confirmation of the reduced level of free glutamic acid in the rat brain during thiopentone anaesthesia was obtained by observation of amino-acid chromatograms of brain extracts. Samples of extracts prepared from normal and anaesthetized litter mates were spotted as pairs on filter-paper sheets and subjected to chromatography with phenol. After development of the chromatogram with ninhydrin, eight observers were asked to choose, from the intensity and area of the colour, which 'glutamic acid' spot of a given experimental pair contained the more amino-acid. The identity of the spots was unknown to the observers and if no difference could be detected between the spots the result was not recorded. Table 3 records the results obtained by the

Table 3. *Examination of the 'glutamic acid' spot on one-dimensional chromatograms of brain extracts from control and anaesthetized rats*

(Rats injected intraperitoneally with thiopentone sodium (60 mg./kg.) and killed after 1.2-1.7 hr. anaesthesia.)

Visual observation of 'glutamic acid' spot*

Experimental pair no.	Control	Anaesthetized
1	8	0
2	5	1
3	7	0
4	2	1
5	8	0
6†	7	0
7	8	0

* Each experimental pair of spots was judged for greater amino-acid content by eight observers. Columns record the number of these observers who considered that one spot of an experimental pair contained more amino-acid than the other.

† 7 observers.

visual observation of seven pairs. In two pairs (nos. 4 and 5, Table 3) estimations of the amino-acid nitrogen of these spots were made; the values, in mg. N/100 g. brain, were 19.8 and 20.2 for the control animals and 12.4 and 11.0 for the anaesthetized animals. These results support the conclusions reached from the microbiological estimations.

Thiopentone inhibition of brain respiration. As the possibility exists that the reduced glutamic acid content of the rat brain during thiopentone anaesthesia is connected with the lowered glucose utilization of the brain, it was decided to investigate the effect of the drug on the respiration of isolated brain tissue with various substrates. It was found that the oxygen consumption of whole rat brain which had been minced and suspended in a glucose-Ringer solution was reduced by low concentrations of thiopentone (Table 4). Thiopentone as the free thiobarbituric acid is only slightly soluble in water, and measurements showed that the maximum

solubility in a phosphate buffer of pH 7.4 at 37° was not greater than 0.015 %. It was found that on the addition of thiopentone suspensions to produce a concentration higher than 0.015 % the respiration of minced brain tissue was still further reduced. A possible explanation of this result is that there is an

Table 4. *Inhibition of the respiration of minced rat-brain tissue by thiopentone*

(Results are the mean of two determinations. Each Warburg vessel contained 200 mg. minced brain in 1.8 ml. Krebs phosphate-Ringer solution. Glucose concentration 0.015M. Temp. 37°.)

Thiopentone added (%)	Inhibition of respiration during 1st hr. (%)
0.08	21
0.04	19
0.02	15
0.01	10
0.005	8
0.001	0

absorption of thiopentone into the lipids of brain tissue. The inhibition by thiopentone of the respiration of isolated rat-brain tissue has also been determined by Fuhrman & Field (1943). These workers investigated the inhibition over a somewhat higher range of drug concentrations, the narcotic being added as the alkaline solution of the sodium salt. They found that at high concentrations (0.15 %) of thiopentone the respiration of rat-brain slices was inhibited by about 80 %.

The oxidation of glutamic acid by brain tissue is known to be inhibited by low concentrations of barbiturates (Quastel & Wheatley, 1932). Table 5 records the effect of 0.04 % thiopentone on the stimulation of respiration produced by adding glutamate to minced brain tissue, after 3 hr. of previous autoxidation. In the initial stages of this autoxidation without any added substrate, the oxygen uptake of minced brain tissue was reduced by thiopentone. However, after 2 or 3 hr. the respiration was not inhibited by the drug and was sometimes even slightly higher than that of the control.

The oxidation of α -ketoglutaric acid by minced rat-brain tissue was also found to be inhibited by thiopentone (Table 5). When, however, glucose was added to minced brain tissue which had been allowed to autoxidize for 3 hr. the resulting stimulation of respiration was almost unaffected by the presence of thiopentone (Table 5). In experiments in which glucose was present from the beginning the respiration was still inhibited by thiopentone during the fourth hour (Table 6), the magnitude of the inhibition being usually increased, as is common with other barbiturates (Jowett & Quastel, 1937). It would appear, therefore, that the preliminary autoxidation of the brain tissue affects the oxidative

Table 5. *Inhibition by thiopentone of oxidations performed by minced rat-brain tissue after autoxidation for 3 hr.*

(All results are the mean of two determinations performed on different rat brains. Each Warburg vessel contained: 1 ml. phosphate buffer pH 7.4; 0.8 ml. Krebs phosphate-Ringer solution with high K content (final concentration 0.015 M); 200 mg. minced brain. Substrate 0.3 ml. in side arm, previously brought to pH 7.4 with NaOH. Centre cup: 0.2 ml. 5% KOH. Temp. 37°.)

Substrate	Thiopentone added (%)	Inhibition in 1st hr. in absence of added substrate (%)	Inhibition in 1st hr. in presence of added substrate (%)	Inhibition in 2nd hr. in presence of added substrate (%)
Glutamate (0.03 M)	0.04	22	42	41
α -Ketoglutarate (0.03 M)	0.04	23	34	32
Glucose (0.03 M)	0.04	21	3	-5

Table 6. *Typical experiment showing the effect of thiopentone and glutamate on the respiration of minced rat-brain tissue*

(200 mg. minced rat brain in 1.8 ml. of Krebs phosphate-Ringer solution. Temp. 37°.)

Substrate	Oxygen consumed (μ l.)			
	1st hr.	2nd hr.	3rd hr.	4th hr.
None	227	152	92	54
Glucose (0.015 M)	260	234	197	155
Glucose (0.015 M) + sodium glutamate (0.005 M)	275	234	176	116
None + 0.04% thiopentone	162	132	88	53
Glucose (0.015 M) + 0.04% thiopentone	205	189	148	105
Glucose (0.015 M) + sodium glutamate (0.005 M) + 0.04% thiopentone	205	169	132	86

systems so that the thiopentone-inhibited step is no longer a limiting factor of glucose oxidation.

In some further experiments the respiration of minced rat brain tissue was measured with a substrate of glucose (0.015 M) plus glutamate (0.005 M). It was found that the addition of thiopentone caused a degree of inhibition of the oxygen uptake which was approximately the same as when glucose was the only substrate. The presence of the glutamic acid slightly accelerated the oxygen uptake during the first hour, but the rate of fall of the respiration was then faster in the presence of glutamic acid and glucose than with glucose alone (Table 6). Dickens (1946) has observed that the presence of glutamic acid accelerates the poisoning of brain slices by high oxygen pressures. It is therefore possible that glutamic acid can also accelerate the oxygen poisoning of minced brain tissue by air.

The effect of thiopentone on the glutaminase and adenylic deaminase of rat brain. The reduced ammonia content found in the brains of anaesthetized rats suggested the possibility of an interference with enzyme systems which can produce ammonia in brain tissue. It was found that the spontaneous liberation of ammonia in buffered extracts of whole rat brain was unaffected by thiopentone (Table 7). The hydrolysis of glutamine by the glutaminase present in rat-brain extracts was also not inhibited by thiopentone (Table 7). The deamination of adenylic acid by brain extracts was in all experiments slightly reduced on the addition of 0.04% thiopentone (Table 7). The inhibition

appeared to increase with time and was also shown when the incubation temperature was 17 or 30°.

Table 7. *Effect of thiopentone on some enzyme systems in brain capable of producing free ammonia*

Enzyme system	Thiopentone added (%)	Inhibition during 1st 30 min. (%)
Brain extract: spontaneous NH_3 formation	0.04	0
Glutaminase of brain extract	0.04	0
Adenylic deaminase of brain extract	0.04	14*

* Mean of three determinations on different rat brains.

Initial results show that adenylic deaminase of brain is also poisoned by barbitone, although urethane has no action on the enzyme.

DISCUSSION

The present results clearly indicate that the free glutamic acid content of the brain is reduced in rats anaesthetized with thiopentone. The estimations of glutamic acid were made after decapitation of the animals and the rapid transference of the head to liquid oxygen to stop the post-mortem biochemical activity. Unless it is believed that the difference in the glutamic acid level became apparent in the brief interval between death and the termination of enzymic activity the change described must be regarded as having occurred *in vivo*.

During anaesthesia it has been shown that the consumption of oxygen by the brain *in vivo* is lowered (Himwich *et al.* 1947), and it seems possible that the reduction of the glutamic acid content is associated with this depressed metabolism. Thus, glutamic acid is closely connected with the oxidative degradation of glucose through the glutamic dehydrogenase system. It has been suggested that one of the physiological functions of glutamic acid in brain is to act as a metabolic buffer of the tricarboxylic acid cycle, assuring an optimal functional rate of the cycle at the α -ketoglutaric acid step (Waelsch, 1949).

It is also possible that a depression of the respiration would allow an actual loss of glutamic acid from the brain to the blood stream. In this connexion it has been shown by Stern, Eggleston, Hems & Krebs (1949) that the maintenance of a high glutamic acid content in isolated slices of brain is dependent upon respiration and energy-giving reactions.

In considering these possible explanations of the reduced glutamic acid level in the brain during thiopentone anaesthesia it becomes desirable to know more about the mechanism by which the barbiturates produce their narcotic activity. It has been suggested by Quastel & Wheatley (1932) that barbiturates cause anaesthesia by an actual inhibition of the oxidations occurring in nervous tissue. This theory, and the alternative viewpoint that the reduced metabolism of the nervous system is a secondary result of the depression of nervous activity, have recently received considerable attention (Gerard, 1947; Butler, 1950).

From the present measurements it can be concluded that the respiration *in vitro* of minced rat brain tissue can be inhibited by low concentrations of thiopentone. In some further experiments it was found that an injection of 30 mg. sodium thiopentone/kg. would just lightly anaesthetize young rats. Assuming the drug was equally distributed through the soft tissues of the body (Butler, 1950) the concentration of thiobarbiturate in the brain *in vivo* would only just inhibit the respiration of minced brain tissue *in vitro*. However, the drawing of definite conclusions from these results is prevented by a consideration of the different conditions under which the brain respire *in vitro* and *in vivo*. Moreover, the possibility of localized regions of barbiturate concentration and inhibition in the brain *in vivo* cannot be excluded. Additional evidence which bears on this problem is provided by the observation that the level of high-energy phosphoric esters in the brain actually increases during barbiturate anaesthesia (Stone, 1940; Dawson & Richter, 1950b). This observation is not consistent with the theory that the actual cause of anaesthesia is a primary inhibition of brain oxidations, if it is assumed that carbohydrate metabolism is related to

nerve function through the energy-rich phosphoric esters (Dawson & Richter, 1950b; Buchel & McIlwain, 1950).

It seems unlikely, therefore, that the fall of the glutamic acid level in the brain occurring during thiopentone anaesthesia is connected with a physiological necessity for the production of energy-rich phosphate bonds. If the glutamic acid lost is metabolized by oxidation it must be considered as a response of the reversible glutamic dehydrogenase system to the reduced production of α -ketoglutaric acid by glucose degradation.

The reduced ammonia content of the anaesthetized brain found in the present investigation and in previous work (Richter & Dawson, 1948) may be associated with the slightly raised glutamine concentration. The synthesis of glutamine is known to require high-energy phosphate bonds (Elliott, 1948), which are present in the brain during anaesthesia at an even higher concentration than normal. Glutamine hydrolysis is not likely to occur in response to the reduced glutamic acid level, as brain glutaminase shows little activity at a physiological pH (Krebs, 1935). The present observations also indicate that the enzyme in the brain deaminating adenylic acid can be poisoned *in vitro* at a low concentration of thiopentone. This result is, however, of doubtful physiological significance as it was shown by Kerr (1942) that measurable free adenylic acid was present in the brain only after some minutes of post-mortem enzymic activity.

SUMMARY

1. The free glutamic acid content of rat brain estimated after decapitation is reduced in animals anaesthetized with thiopentone.

2. The glutamine content of the brain is slightly increased in anaesthetized rats, but this accounts for only part of the fall in the glutamic acid level.

3. Maintenance of the body temperature of the rats during narcosis decreases the amount by which the glutamic acid level in the brain is reduced.

4. The glutamine and glutamic acid levels in the rat brain are not significantly changed from the normal values by alteration of the environmental temperature or by electrical stimulation of the brain.

5. The inhibition of the respiration of minced rat brain tissue by low concentrations of thiopentone has been measured.

6. Thiopentone anaesthesia reduces the level of free ammonia found in the rat brain.

7. Adenylic deaminase present in brain extracts is slightly poisoned by 0.04% thiopentone, while glutaminase is unaffected.

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The Composition of some Microbial Deoxypentose Nucleic Acids

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Recent advances in nucleic acid chemistry, principally through the application of chromatographic techniques, have led to the abandonment of the tetranucleotide hypothesis and the discovery that both deoxypentose nucleic acids (DNA) and ribonucleic acids (RNA) show a range of compositions characteristic of their source. Because of the close association of DNA with the chromatin material of the nucleus, comparative studies on the structure of DNA from different organisms have a special biological significance. Analyses by Chargaff and co-workers have led to the conclusion that, among animals, the composition of DNA (i.e. the molar proportions of the purine and pyrimidine bases) is characteristic of the species source and constant for different tissues of one species, and that DNA from bacteria also shows characteristic differences in composition (Chargaff, 1950; Chargaff, Zamenhof, Brawerman & Kerin, 1950). Results obtained in this laboratory with improved chromatographic techniques and methods of hydrolysis, while in general agreement with these conclusions, have also led to the recognition of the pyrimidine, 5-methylcytosine, as a constituent of DNA from animals and higher plants; this is apparently lacking from nucleic acids of microbial origin (Wyatt, 1950, 1951a, b). It is

apparent that in molecules so large as those of the nucleic acids many possibilities exist for structural differences which do not involve differences in nucleotide composition; nevertheless it is only through such quantitative analysis that an approach may at present be made to the comparison of different nucleic acids. We have therefore extended these analyses to include some bacterial and virus deoxypentose nucleic acids, and shall now describe the determination of the purine and pyrimidine composition of DNA from *Mycobacterium tuberculosis*, *Escherichia coli*, the two *E. coli* bacteriophages T₂ and T₆, and the virus causing polyhedral disease in gipsy-moth (*Lymantria dispar* L.) caterpillars.

It is now recognized that all bacteria which have been examined contain both RNA and DNA, and there is evidence that the DNA is confined to the bacterial nucleus (Robinow, 1945). *E. coli*, which contains about 4% (dry wt.) of DNA, was chosen in order to permit comparison of the nucleic acids in a virus-host system. The tubercle bacillus was of particular interest because of the reported presence of 5-methylcytosine in its nucleic acid (Johnson & Coghill, 1925), although Vischer, Zamenhof & Chargaff (1949) had been unable to confirm this claim.

The bacteriophages and the polyhedral viruses of insects are convenient for nucleic acid analysis

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