strain) were used. Litter mates were divided into 2 equal groups for experimental and control animals. A total of 24 males and 24 females was used for each group. The experimental animals were exposed to carbon monoxide, by a method previously described,[6] 8 hours daily, except Saturday and Sunday.

Results. Sixteen of the control females have borne litters; 4 of them have borne 2 litters each. Of the 20 litters, 16 were weaned, averaging 5.0 to a litter (all litters were reduced to 6 at birth). This is somewhat below normal fertility, but the rats were taken from a newly established colony, unselected for breeding. During this same period there were 15 pregnancies in the experimental group. Four of these terminated in abortion and 11 litters were delivered at term. It was impossible to determine accurately the number of young in each litter, because the mother usually ate some of the young soon after birth. The average litter size (including stillborns) was 3.9. The average weight of the young was 3 to 5 g, while the average weight for the young of control animals was 5 to 7 g. In every case the mother refused to nurse her young, and death of all the young usually occurred in 1 to 3 days; the longest survival period was 8 days.

The growth curves for experimental and control animals were identical within limits of normal variation. At 114 days the average weights were: male controls 310 g, male experimentals 300 g, female controls 176 g, female experimentals 179 g. These results differ from those of Campbell,[2] in which much higher concentrations of carbon monoxide were used. He observed that during the first 100 days, when acclimatization was developing, the mortality rate was high and growth lagged behind that of the controls. Killick[4] observed a loss of weight in adult mice during acclimatization to gradually increasing concentrations of carbon monoxide.

Discussion. Our results emphasize the importance of Campbell's[2] insistence that acclimatization to carbon monoxide (or to any form of anoxia) cannot be considered complete unless fertility (pregnancy leading to the birth of normal young) is unimpaired. We would like to suggest, tentatively, that normal lactation leading to the weaning of healthy young be included, since, in at least one of our cases, a litter of normal size and average weight failed to nurse after 5 days and died in 8 days. Further studies are needed to determine the cause of the invariable failure of mothers exposed to carbon monoxide to nurse their young.

Conclusions. Rats exposed daily to carbon monoxide (0.04%) grow normally and have about the same incidence of pregnancy as do control litter mates. However, there is a tendency toward abortion, the average size of the litter and the average weight of the young at birth are subnormal, and the mothers invariably fail to nurse their young. The cause of this lactation deficiency is under investigation.

[6] Miller, A. T., Jr., *J. Lab. and Clin. Med.*, 1943, **28**, 1854.

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Acetylation in Patients with Myasthenia Gravis. The Elimination of Para-amino Benzoic Acid.

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The weakness of certain skeletal muscles and the easy fatiguability observed in patients with myasthenia gravis is probably due to an insufficient production of acetylcholine (Torda and Wolff[1]). This decreased synthesis of

[1] Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 224.

TABLE I.
Short Summary of the Clinical State of the Patients with Myasthenia Gravis.

Name	Sex	Age	Severity of disease	Duration (years)	Thymectomy	X-ray treatment	Symptomatology	Neostigmine (Prostigmine bromide) (Hoffmann-LaRoche) Dose before thymectomy mg/day	Dose mg/day	Achievement after medication
H	F	43	5+	15	yes	yes	Bedridden, severe lid ptosis, diplopia, constant difficulty in chewing and swallowing, very severe muscular weakness	225	120-180	Chews food, turns in bed, sits on chair
R	F	23	3+	9	no	"	Moderate lid ptosis, occasional diplopia, occasional difficulty in chewing, moderate muscular weakness		90	Walks 1-2 blocks
Sa	F	32	3+	7	"	"	Moderate lid ptosis, occasional diplopia, occasional difficulty in chewing, moderate muscular weakness		90-150	Housework
S	M	22	2+	2	yes	no	Slight lid ptosis, rare diplopia, moderate muscular weakness	120	15-45	Works as an inspector
N	F	47	1+	12	no	"	Mild muscular weakness		30	Housework

acetylcholine may be due to one or more factors. Insufficient amounts of basic precursor substances, namely, choline and the acetyl- radical, is one of these possible factors. The purpose of the following investigation is to ascertain whether processes of acetylation are defective in patients with myasthenia gravis. Since acetylation of choline and acetylation of other chemically different substances have probably in common only the use of the acetyl- radical, faulty acetylation of other substances than choline would indicate that insufficient amount of acetyl- radical was available. Therefore, if an agent were administered that was eliminated in acetylated form in healthy subjects, the success or failure of patients with myasthenia gravis to achieve acetylation would be an indication of the ability of the patients to complete such a process. From the group of substances which may be acetylated by man if taken by mouth, *p*-amino benzoic acid[2-6] was selected for this investigation.

Method. *P*-amino benzoic acid was administered orally in both small and large doses to both healthy subjects and to patients with myasthenia gravis, and the elimination of free and acetylated *p*-amino benzoic acid was determined. Summaries of the clinical state of the patients with myasthenia gravis are presented in Table I.

I. *Small Doses.* 300 mg of *p*-amino benzoic acid were orally administered in a single dose to healthy subjects and to patients with myasthenia gravis. Separate specimens of urine were collected over a period of 24 hours. The free and conjugated *p*-amino benzoic acid content of the separate samples were ascertained following the method of Bratton and Marshall.[7]

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TABLE II.
Excretion of 300 mg *p*-Amino Benzoic Acid.

Hr after ingestion	Control subjects								Patients with myasthenia gravis									
	G	T_1	W	T_2	Su	Be	Si	La	We	Wo	Me	T_3	H_1	H_2	R	S	N
									Free *p*-amino benzoic acid									
2	25.0	7.7	19.0	13.3					14.9		15.7	12.0		16.0			
3																15.0	
4	1.2	6.3	3.8	11.3	18.0		20.0		12.6	41.0		3.7					11.4
6	1.0	7.0	0.2	3.4				25.0			18.4			14.3	26.0		
8	0.5	2.5	1.0			17.2	12.0		0.9			1.5	36.2				4.6
10	0.5	0.2	0.4	0.8			2.4	2.0					2.0	2.2		6.8	
12		0.6	0.2									2.8	1.2	0.4			0.7
16	0.8						4.2	3.0									0.9
22		1.1	0.3	0.9	6.0	6.7		4.5						1.2		1.9	2.0
Total	29.0	25.4	24.9	29.7	24.0	23.9	38.6	34.5	(28.4)	(41.0)	(34.1)	20.0	39.4	34.1	(26.0)	23.7	19.6
									p-Acetylamino benzoic acid									
2	42.0	41.9	36.2	19.5					62.0		34.0	31.2		39.0			
3																46.0	
4	86.0	83.0	52.6	35.4	104.0		105.0		57.0	130.0		75.6					69.0
6	44.5	36.2	37.0					161.0			80.0			77.0	154.0		
8	11.6	6.4	34.0	94.2		148.8	49.8	43.6	33.7			52.5	119.5				72.0
10	3.9	8.0	14.2	20.7			19.2						34.0	50.0		133.0	
12		2.0	5.0	8.0								40.5	34.0	6.6			35.0
16	6.7						15.5	4.0									6.8
22		3.5	3.5	3.7	96.2	30.4		3.0				10.0		0.3		12.0	2.8
Total	194.7	181.0	182.5	181.5	200.2	179.2	189.5	211.6				209.8	187.5	172.9		191.0	185.6

TABLE III.
Excretion of 2 g *p*-Amino Benzoic Acid.

Subject	Excreted in mg	
	p-amino benzoic acid	*p*-acetylamino benzoic acid
Healthy:		
T_1	1000	680
T_2	1120	580
Myasthenia gravis:		
Sa	876	684
R	1085	650

II. *Large Doses.* 2 g of *p*-amino benzoic acid were orally administered in a single dose to both healthy subjects and patients with myasthenia gravis. Urine was collected over a period of 48 hours. An aliquot was taken to ascertain the amount of free and conjugated *p*-amino benzoic acid excreted (method of Bratton and Marshall[7]). The conjugated *p*-amino benzoic acid was isolated from the remainder following the method described by Harrow and Mazur[8] and the identity of the isolated compound with *p*-acetylamino benzoic acid was ascertained by determination of the melting point.

Results. I. *Excretion of Small Doses of* p-*Amino Benzoic Acid.* Seventy per cent of the 300 mg of *p*-amino benzoic acid taken in a single dose was excreted during the first 24 hours. On the average 45% was excreted in conjugated form and the remaining in free form as shown in Table II. Both healthy subjects and patients with myasthenia gravis excreted *p*-amino benzoic acid in essentially the same manner in respect to time of elimination and amount of free and conjugated compound.

II. *Excretion of Large Doses of* p-*Amino Benzoic Acid.* Eighty per cent of the 2 g of *p*-amino benzoic acid were excreted during the first 48 hours. On the average 34% was excreted in conjugated form and the remainder in free form as shown in Table III. Patients with myasthenia gravis excreted this large dose of *p*-amino benzoic acid in a manner similar to healthy persons. However, it seemed to be necessary to ascertain whether the conjugated compound was *p*-acetylamino benzoic acid in both cases. Therefore the compound was isolated and the melting point determined. Since the melting points of the isolated compounds were 248°C in both cases, it is likely that *p*-amino benzoic acid was eliminated as *p*-acetylamino benzoic acid in both healthy subjects and patients with myasthenia gravis.

Discussion. Both patients with myasthenia gravis and healthy subjects excreted *p*-amino benzoic acid in essentially the same manner in respect to time of excretion and amounts of free and conjugated (acetylated) form. A faulty acetylation resulting from insufficient acetyl- radical would be more easily detected with large doses of *p*-amino benzoic acid taken because of the increased requirement. Since both healthy subjects and patients with myasthenia gravis excreted similar amounts of *p*-amino benzoic acid, it may be assumed that there is no significant insufficiency of the acetyl- radical in patients with myasthenia gravis.

The acetylation of *p*-amino benzoic acid is not necessarily a measure of the acetylation of choline, and probably involves entirely different processes. The acetylation of *p*-amino benzoic acid occurs mainly in the liver[6] while the acetylation of choline occurs mainly in the nervous tissue. The enzymes involved and the oxygen requirements of the two processes may also differ. However, an undisturbed and ready acetylation of *p*-amino benzoic acid even in large amounts suggests that no severe insufficiency of the acetyl-radical occurs in patients with myasthenia gravis. A similar conclusion may be drawn from the clinical observation that patients with myasthenia gravis do not benefit significantly from the administration of insulin and glucose.

Summary. 1. The excretion of both small and large amounts of *p*-amino benzoic acid

[8] Harrow, B., Mazur, A., and Sherwin, C. P., *J. Biol. Chem.*, 1933, **102**, 35.

administered orally in a single dose to healthy subjects and to patients with myasthenia gravis was investigated. 2. Both healthy subjects and patients with myasthenia gravis excreted *p*-amino benzoic acid in essentially the same manner in respect to time of elimination and amounts of free and acetylated form. 3. It is inferred that there is no serious insufficiency of acetyl- radical in patients with myasthenia gravis.

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Action of Sulfonamides on Toxins of Agents of the Lymphogranuloma-Psittacosis Group.

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It has been shown elsewhere[1,2,3] that all four of the agents of the lymphogranuloma-psittacosis group[4] investigated so far produce a rapidly lethal toxin which in general resembles the bacterial endotoxins. These toxins are distinguishable one from the other by certain characteristics of the distribution curve of time of death and by differences, in certain instances, in the pathological picture produced, as well as by the sharp specificity of the toxin-antitoxin reaction. While intravenous inoculation of the agent of lymphogranuloma venereum results only in early deaths[1,2] that could hardly be due to anything other than toxin (since 83% of deaths occur within 24 hours and 95% within 36), the other 3 toxic materials produce diphasic distribution curves of time of death, the early curve of toxicity being followed by a later curve in which deaths are due to infection.[2,3]

Following the earliest report on the existence of these toxins the question has been raised as to how one could be sure that one was dealing with a toxin and not with an infection which was acting very rapidly. This point, while hardly valid in connection with the very early deaths, did seem to be pertinent in those cases where toxemia was followed, often without any distinct interval (*i.e.*, in the case of meningopneumonitis), by deaths certainly due to infection. Could one distinguish clearly between the two forms of death?

It has been possible to demonstrate that infection with 2 of these agents, namely, lymphogranuloma venereum and mouse pneumonitis, is controllable with sulfonamides[5,6] while the infection with feline pneumonitis (Baker[7,8]) and meningopneumonitis is not.[3,6] If the theories, which have been postulated elsewhere[1,2,3] and set out briefly above, concerning the cause of deaths following intravenous inoculation of mice with these toxins are correct it should be possible to demonstrate this fact by means of sulfonamide therapy. In the case of lymphogranuloma venereum only supposedly toxic deaths occurred and these should be unaffected by sulfonamides. In the case of mouse pneumonitis where there is a distinct interval between the early, supposedly toxic, deaths and the later infectious deaths the later deaths should be eliminated by sulfonamide therapy while the early deaths should not. Finally, meningopneumonitis and feline pneumonitis both give diphasic curves of death and the infectious deaths follow closely upon the toxic. Infections due to these agents are not affected

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