Oral Toxicity and Lipotropic Potency of the Triethyl Homologue of Choline

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Interest in the triethyl homologue of choline arose in 1936 before tracer elements were available as aids in the study of the intermediary metabolism of compounds of physiological interest. Channon & Smith (1936) desired to test the hypothesis that dietary choline exerts its lipotropic effect by favouring the synthesis of lecithin, which in turn might speed up the transport of fat from the liver. They sought a compound resembling choline which would possess lipotropic properties and which might, if incorporated into the molecule of liver lecithin, be identified. Several homologues of choline were prepared and tested for ability to prevent the excessive accumulation of fat always seen in the livers of rats on hypolipotropic diets (Channon, Platt & Smith, 1937). Most of these compounds exhibited considerable toxicity when given by mouth, but one of them, triethyl-2-hydroxyethylammonium chloride (hereafter referred to for brevity as TECl) could be given to rats in sufficient amount to produce the hoped for decrease in liver fat. Males did not always respond as well as females and were, in some cases, relatively unresponsive.

Although TECl had been shown to possess definite lipotropic activity, Channon, Platt, Loach & Smith (1937) were unable to detect the presence of this homologue of choline in the liver phospholipids of rats given about 11.5 mg. daily for 20 days. Higher dosage was avoided because of the toxicity of the compound. Ten years later the validity of their attractive hypothesis received support when McArthur (1946) succeeded in establishing the presence of the triethyl homologue of choline in the liver phospholipids of rats fed TECl. Meanwhile, the lipotropic activity of TECl had been confirmed and the absence of lability of the terminal ethyl groups was established, as expected, when TECl was found to be incapable of supporting the growth of young rats on diets containing homocystine as the only source of amino-acid sulphur (du Vigneaud, Chandler, Moyer & Keppel, 1939; Moyer & du Vigneaud, 1942). TECl was found to prevent the development of haemorrhagic kidneys in young rats (Welch, 1941), but it does not protect poultry against perosis or permit their growth (Jukes, 1941).

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McArthur, Lucas & Best (1947) described in detail the conditions under which the incorporation of TECl into liver phospholipids was established. Their rats were given about six times as much of the choline homologue per day for twice as long a period without occasioning as much loss of body weight as was found by Channon, Platt, Loach & Smith (1937).

Studies of the acute and chronic oral toxicity of TECl (made before the experiments of McArthur *et al.* were undertaken), lipotropic dose-response curves of TECl and of choline chloride, hitherto unpublished, and protocols of an experiment in which weanling rats were given TECl in place of choline chloride are presented briefly in the following pages.

EXPERIMENTAL

TECl used in the present studies was made in a yield of 60–75% from carefully redistilled ethylene chlorohydrin (British Drug Houses, fraction boiling at 127–128°) and triethylamine (Eastman Kodak, fraction boiling at 88–89°) as described in detail elsewhere (McArthur *et al.* 1947). After recrystallization from absolute ethanol (0.5 ml./g.), acetone (1 ml./g.) and anhydrous ether (5 ml./g.) the product was tested for contamination with triethylamine hydrochloride by boiling a portion in the Parnas-Wagner micro-Kjeldahl apparatus with strong NaOH. If any volatile base distilled over in 3 min., the product was recrystallized.

Acute oral toxicity was determined by giving the TECl (generally in 1.00 ml. of solution) by stomach tube. Rats of both sexes, weighing between 150 and 160 g. were used. In a preliminary test, groups of three rats were given 25, 50, 100 and 200 mg., respectively. One death only, and that in the final group, was obtained. Groups of three rats were then given 400, 600 and 800 mg. of TECl, respectively, as shown in Table 1 and Fig. 1, where all the data from this and subsequent tests are summarized. The effect of concentration, as distinct from absolute amount of drug, was examined by giving some of the test doses in 2.00 ml. Choline chloride was given to other rats under identical conditions. No special diet was fed to the rats used for these acute oral toxicity studies. Food and water were withheld for 1 hr. previous to the test dose and for about 3 hr. following administration of the drug.

The chronic toxicity studies were conducted with groups of five rats (150–170 g.) fed on a diet of the following percentage composition: casein, 8; gelatin, 12; salts (McCollum No. 185), 5; agar, 2; sucrose, 41; 'vitamin powder A', 2; cod liver oil concentrate (Ayerst, McKenna and Harrison), 0.010; beef dripping, 25; corn oil (Mazola), 5. 'Vitamin powder A' was a mixture in powdered sucrose of such a com-

position that at a food intake of 8 g./day each rat would receive aneurin hydrochloride (30 μ g.), riboflavin (25 μ g.), pyridoxin hydrochloride (20 μ g.), Ca pantothenate (100 μ g.) and nicotinic acid (100 μ g.). Three such diets, containing 0.22, 0.45 and 0.89% TECl, respectively, were fed to the rats for 21 days, and subsequently, because of the negligible toxic effects observed, a diet containing 1.13% was fed to 20 rats for 40 days.

A similar basal diet containing 30% beef fat was used to determine the lipotropic dose-response relationship in rats weighing 70–90 g. Two separate experiments were performed with dietary fat content at 30% and one with it at 12% (beef fat 10%, corn oil 2%). A more severely hypolipotropic, but in other respects a more adequate, diet (T45) was used for a similar dose-response study with weanling rats (36–42 g.). Diet T45 has the following percentage composition: arachin, 12; gelatin, 6; casein, 3; fibrin, 1; cystine, 0.5; salt mixture (Beveridge & Lucas, 1945), 5; cellulose, 2; sucrose, 57.5; beef dripping, 10; corn oil 2; cod liver oil concentrate, 0.015; 'vitamin powder B', 1. This mixture (powder B) contained four more vitamins than did powder A. One kg. of vitamin powder B contained aneurin hydrochloride, 0.50 g.; riboflavin, 0.25 g.; pyridoxin hydrochloride, 0.20 g.; Ca pantothenate, 1.00 g.; nicotinic acid, 1.00 g.; folic acid, 0.05 g.; 2-methyl-1:4-naphthoquinone, 0.10 g.; inositol, 50 g.; *p*-aminobenzoic acid, 10.0 g. and powdered sucrose, 936.9 g.

The supplements added to these basal rations and the results are shown in Tables 2–4 and Fig. 1. It should be noted that the supplements of TECl and choline Cl were added in amounts molecularly equivalent to 2, 4, 8, 16, 32 or 64 mg. of choline (free base) per 10 g. of diet.

The rats, housed in all-metal individual cages with a false bottom of coarse wire screen, were supplied with fresh water *ad lib.* and were offered 8.0 g. of food daily; unconsumed diet was weighed each morning. The daily food consumption was recorded and the TECl or choline Cl intake per rat calculated. At the end of the experimental period the animals were stunned and the livers and kidneys were removed and weighed. When portions of these organs were taken for histological examination the remainders were reweighed and disintegrated immediately under acetone in a pyrex test tube (100 ml.) by means of a rapidly revolving micromincer, somewhat similar to one described by Alford & Palmes (1944). Best, Lucas, Patterson & Ridout (1946) have already described the extraction of total lipids with boiling ethanol, their rectification with light petroleum, and the analytical procedure used in this laboratory.

RESULTS

The data in Table 1 and Fig. 1 show that the acute toxicity of TECl administered orally to rats is slightly greater than twice that of choline chloride given under similar conditions. The effects of TECl or of choline chloride solutions administered by stomach tube were apparent, in most cases, within 15 min. Both compounds in adequate doses produced similar states of hyperexcitability, often including convulsions, followed by collapse, respiratory difficulty and eventually death within 30 min. The animals that survived frequently failed to exhibit the excitement; they appeared drowsy and

remained quiescent unless disturbed. Only in a few cases did animals that survived the initial 30 min. period die within the following 16 hr. We observed, as has already been noted by Neuman & Hodge (1945), a marked difference in susceptibility of groups of rats tested at different times. For example, in February 1946, three out of three rats died on oral administration of 150 mg. of TECl in 1 ml.; in March 1948 none out of six rats died from a similar dosage.

In Table 1 are summarized the results of toxicity studies made with small groups of rats at five different times, over a period of more than 3 years. Any changes in tolerance of the population of the rat colony were probably evened out by tests over such a period.

The empirical probits corresponding to the data shown in the first six columns of Table 1 when plotted (Fig. 1) were too irregular for visual estimation of the line of best fit. The method of least squares was therefore used to determine the parameters of this provisional line from which the expected probits (*Y*) shown in column 8 were obtained. The method of estimating the probit regression equation by a process of successive approximations, starting with the provisional probit line, is described by Finney (1947). For the computations required to fit the probit regression line by the maximum likelihood method, the instructions given by Finney (1947, Appendix 1) were followed.

The LD₅₀ of TECl appears to be 316 mg. when administered in 1 ml. of solution by stomach tube to rats weighing 150–170 g. (Dr B. Mendel and Mr D. K. Myers in this Department have estimated the LD₅₀ of the same sample of TECl by intravenous and intraperitoneal injection to be about one-fiftieth of the value found when the drug was given by stomach tube. Details of their work will be published elsewhere.) Choline chloride under similar conditions has an LD₅₀ of about 760 mg. when given in 2 ml. of solution.

No signs of chronic toxicity of TECl were obvious at the dosages studied. The average food consumption of the rats during the first 4 days on the three diets (7.8, 7.5 and 7.0 g., respectively) did not indicate that the diets were unpalatable. Although there was a gradual decline in food consumption, a similar decrease occurred in the control group on the drug-free basal diet. Since there was no progressive decrease in food intake or change in weight which could be related to the TECl content of the diet, it was concluded that even the largest dosage fed was without definite toxic effect. When the diet containing 1.13% TECl was fed to 20 rats for 40 days, a slight loss in weight (about 10%) was observed during the first 20 days, but after that time the weight remained essentially constant. With the food consumption averaging just over 6 g. per day during the 40-day period the daily intake of the TECl was about 70 mg. The failure of these rats to gain in

Table 1. *Estimation of median lethal dose (LD₅₀) of TECl and comparison with that of choline chloride*
(Probit regression line for: TECl, $Y = 2.93x - 2.31$, LD₅₀ = 316 mg.; choline chloride, $Y = 10.04x - 23.97$, LD₅₀ = 760 mg.)

Dose (mg.)	Volume (ml.)	Log dose (<i>x</i>)	No. of rats (<i>n</i>)	Deaths		Probits				Calc. kill (<i>P</i>)	Expected deaths (<i>nP</i>)	Discrepancy (<i>r</i> - <i>nP</i>)
				Obs. (<i>r</i>)	%	Empirical	Expected <i>Y</i>	Working <i>y</i>	Max. likelihood			
TECl												
100	1.0	2.00	3	0	0	—	3.92	3.29	3.55	0.075	0.23	- 0.23
150	1.0	2.18	9	3	33	4.56	4.31	4.58	4.07	0.177	1.62	+ 1.38
200	1.0	2.30	12	3	25	4.33	4.56	4.34	4.42	0.280	3.36	- 0.36
250	1.0	2.40	22	7	32	4.53	4.77	4.54	4.72	0.390	8.58	- 1.58
300	1.0	2.48	24	11	46	4.90	4.94	4.90	4.92	0.470	11.28	- 0.28
350	1.0	2.54	24	13	54	5.10	5.07	5.10	5.13	0.550	13.20	- 0.20
400	1.0	2.60	14	10	71	5.55	5.21	5.56	5.30	0.617	8.64	+ 1.36
600	1.5	2.78	3	2	67	5.44	5.59	5.43	5.83	0.797	2.39	- 0.39
800	2.0	2.90	3	3	100	—	5.83	6.55	6.18	0.880	2.64	+ 0.36
Choline chloride												
600	2.0	2.78	10	2	20	4.16	3.96	4.18	3.94	0.145	1.45	+ 0.55
800	2.0	2.90	9	4	44	4.85	5.25	4.84	5.14	0.555	4.99	- 0.99
950	2.0	2.98	10	9	90	6.28	6.11	6.27	5.95	0.830	8.30	+ 0.70

weight is attributed to the low methionine content, or some other defect, of the basal diet and not to drug toxicity since the animals on the basal diet

Fig. 1. Relationship between dosage of TECl and percentage kill. Observed percentage kill (●) converted to probits (+). The probit regression line was fitted by maximum likelihood method and the sigmoidal curve relating log dose to percentage kill was derived from this line. For comparison, the probit regression line for choline chloride (Δ) is also given.

exhibited similar weight changes. The toxicity observed by Channon & Smith (1936) and Channon, Platt, Loach & Smith (1937) was probably attributable largely to inadequacies of their basal diet.

The lipotropic dose-response curves (Fig. 2) show that the triethyl homologue of choline, although considerably less potent than choline, will, if given in sufficient amount, bring the total liver lipids down to the same normal value as does an adequate amount of choline. The data of curves A-D were obtained under identical dietary conditions, except that the nature and amount of fat were different in the diets fed to the rats whose liver lipids are represented by curve A.

Curve A shows the lipotropic effect of TECl in a diet containing 12% fat (beef fat 10%, corn oil 2%), and curve B represents the mean response from two experiments in which the diets contained 30% beef fat (Table 2). The consistently higher level of liver lipids on the lower fat intake (curve A) may possibly be related to change in environmental conditions rather than to the presence of highly unsaturated lipid (corn oil) along with the beef fat. Curve C, drawn through all experimental points available for TECl, represents the mean lipotropic effect of this compound. Curve D represents the lipotropic effect of choline in a diet containing 30% beef fat. Curves B and D may be compared, since they represent responses obtained simultaneously under strictly comparable conditions (30% beef fat).

Comparison of lipotropic potencies can be made only on the sloping portions of the curves, of course, since it is obvious that once such a curve tends to parallel the horizontal axis all doses beyond the shoulder of that curve will produce the same maximal response. To estimate relative lipotropic potencies the amount of each of the agents necessary to produce the same levels of liver lipids must be compared. For example, from curves B and D it may be seen that the amount of TECl molecularly equivalent to 4 mg. of choline base reduces the liver

Table 2. *Lipotropic activity of TECl*

(Test diets containing 30% beef fat fed for 21 days to groups of 10 rats, initially weighing 75–90 g. All rats group paired-fed with those on basal ration; average daily food intake in Exp. *TA* about 6.7 g.; in Exp. *TB* about 5.6 g. (except *TB-4*, in which it averaged only 4.3 g.)

The supplements were molecularly equivalent to 2, 4, 8, 16, 32 or 64 mg., respectively, of choline (free base)/10 g. of food, e.g. diet *TA-3* containing 12.0 mg. TECl/10 g. and diet *TA-6*, containing 9.2 mg. choline chloride, each contains an amount of quaternary ammonium salt corresponding molecularly to 8 mg. of choline base.)

Group no.	Supplement in terms of choline equiv. (mg./10 g.)	Average liver wt.		Total liver lipids (g./liver)	Kidney	
		Wet (g.)	Dry, fat-free (g.)		Wt. (g.)	Body wt. (%)
<i>TA-0</i>	None (basal)	5.14	0.84	1.237	1.23	1.84
<i>TA-1</i>	TECl≡2	4.78	0.85	0.810	1.15	1.60
<i>TA-2</i>	TECl≡4	5.59	0.93	1.164	1.15	1.53
<i>TA-3</i>	TECl≡8	5.02	0.95	0.872	1.03	1.37
<i>TA-4</i>	TECl≡16	4.31	0.85	0.610	0.96	1.41
<i>TA-5</i>	TECl≡32	4.05	0.84	0.316	0.95	1.44
<i>TA-6</i>	Choline Cl≡8	4.09	0.89	0.295	1.12	1.37
<i>TB-0</i>	None (basal)	5.48	0.93	1.364	—	—
<i>TB-1</i>	TECl≡8	5.24	0.94	1.021	—	—
<i>TB-2</i>	TECl≡16	4.86	0.84	0.977	—	—
<i>TB-3</i>	TECl≡32	4.34	0.90	0.287	—	—
<i>TB-4</i>	TECl≡64	3.91	0.80	0.336	—	—
<i>TB-5</i>	Choline Cl≡4	5.20	1.02	0.609	—	—
<i>TB-6</i>	Choline Cl≡8	4.41	0.95	0.323	—	—
<i>TB-7</i>	Choline Cl≡16	4.91	1.06	0.351	—	—
<i>TB-8</i>	Choline Cl≡32	4.62	1.04	0.264	—	—

lipids to 21% wet weight. Choline chloride equivalent to about 0.8 mg. of choline base produced the

TECl on a molecular basis. Since the molecular weights of choline chloride (139.6) and TECl (181.7) are in the ratio of 1 : 1.30, the relative potencies on a weight basis are about 6.5 : 1. Similarly, the amount of TECl equivalent to 8 mg. of choline base has the same effect as choline chloride equivalent to 1.4 mg. choline base, i.e. the ratio (molecular) of potencies is about 6 : 1. However, when the amount of TECl exceeds that corresponding to about 25 mg. of choline base, both curves are approaching the horizontal and valid comparison becomes impossible, as mentioned above.

Channon & Smith (1936) reported that TECl is almost as potent as choline in its lipotropic effect upon female rats but that its effect is far less pronounced on bucks. The data presented here in Fig. 2 and Table 3 fail to confirm either statement. It is now obvious why Channon & Smith mistakenly concluded that choline and its triethyl homologue possess about equal activity: in the absence of a dose-response curve, the drugs were compared at dosage levels beyond that at which they exert their maximal effect, i.e. on the horizontal portion of the dose-response curve (in regions to the right of the arrow in Fig. 2). Possibly because of a difference in the strains of rats used in Liverpool and Toronto, or possibly because of differences in the nature of the basal diet, no significant difference in the response of males and females has been observed, as shown by the data in Table 3.

The effects of three different dietary levels of TECl upon weanling rats are shown in Table 4. Rats

Fig. 2. Lipotropic dose-response curves. Effects of TECl (curves *A*, *B* and *C*) and of choline chloride (curve *D*) on total liver lipids when fed for 21 days. Ten rats (70–90 g.) per group. Lipid values expressed as percentage of wet tissue weight. Curve *A* ($\square$), rats on diet containing 12% fat (beef fat 10%, corn oil 2%). Curve *B*, two separate experiments (+ and $\bullet$) with rats consuming diets containing 30% beef fat. Curve *C* represents the mean of all values obtained with TECl, since it is questionable whether curves *A* and *B* differ significantly. Data for curve *D* ($\triangle$), showing effects of choline chloride in diet containing 30% beef fat, are comparable with those shown by +, obtained at the same time.

Table 3. *Lipotropic response of male and female rats to TECl*

(Ten male and ten female rats (70–90 g.) fed with a diet containing 0.25% TECl for 21 days. Average food consumption of both groups was 6.2 g./day. Values arranged in increasing order.)

Total liver lipids (% wet wt.)		
	Females	Males
	6.9	5.5
	7.2	9.2
	7.6	10.6
	8.0	12.5
	10.4	14.1
	12.6	14.9
	14.2	18.8
	15.2	20.0
	19.1	21.1
	23.2	22.3
Mean	12.4	14.9
S.D.	±5.5	±5.6

in the diet. This confirmation of the statements in the literature may not be as unambiguous as it appears. The lipotropic effects observed in the rats of groups *TC-2* and *TC-3* may be artefacts, since fatty livers and haemorrhagic renal lesions do not develop unless an adequate intake of food is maintained. The reason for the decreasing food intake of these weanling rats consuming diets containing such relatively small amounts of TECl is not known. Certainly the decreasing food intake was not due to kidney failure, since these organs were normal; nor was it likely to be due to toxicity, since older rats can eat diets containing about four times as much as the highest dosage given these weanlings. There is a more acute need for choline *per se* as a structural element in younger rats than in older ones; failure of growth may possibly be the explanation for the apparent lipotropic properties of TECl when fed to weanling rats. The paired-feeding experiments required to settle this point will be undertaken shortly.

Table 4. *Comparison of TECl and choline chloride in weanling rats*

(Rats weighing 36–42 g. (average 39 g.) fed diet *T45* supplemented as shown below. The number shown under the heading 'kidney lesions' is the number of animals showing grossly swollen and darkened ('haemorrhagic') kidneys.)

Group no.	Supplement (in terms of choline equiv.) (mg./10 g.)	Duration (days)	No. of rats	Change in body wt. (g.)	Daily food intake (g.)	Average liver wt.		Total liver lipids (mg.)	Kidney		
						Wet (g.)	Dry, fat-free (g.)		Wt. (g.)	Body wt. (%)	Lesions
<i>TC-0</i>	None (basal)	9	10	- 1	3.4	3.40	0.576	671	1.18	3.11	10
<i>TC-1</i>	TECl≡8	9	10	+ 1	3.0	2.72	0.489	462	0.89	2.22	2
<i>TC-2</i>	TECl≡16	14	10	+ 1	2.9	2.57	0.600	197	0.77	1.98	0
<i>TC-3</i>	TECl≡32	14	8	- 2	2.5	2.35	0.557	149	0.62	1.68	0
<i>TC-4</i>	Choline Cl≡8	14	5	+ 30	6.0	5.97	1.102	1264	1.05	1.50	0

consuming the hypolipotropic basal ration (*TC-0*) became moribund by the ninth day owing to the severely haemorrhagic condition of their kidneys. Coincidentally with the rapid development of the renal lesions between the fifth and ninth days, the food consumption dropped to almost nothing, the average intake on the first day was 5.7 g., on the last day it was only 1.2 g. From other experiments it is known that the liver lipids of these weanlings reaches a maximum (about 23% fresh liver weight) on the sixth day. As uraemia developed, and doubtless as a result of the decreasing food intake, the liver lipids decreased slightly to the value noted (19.7%) at the time of killing.

A marked decrease in liver lipids and in the incidence of haemorrhagic renal lesions was observed as the TECl content of the ration increased. In striking contrast to the rapid growth occasioned by the choline supplement (cf. group *TC-4*, in which the rats almost doubled their weight in 2 weeks) was the complete failure to grow when TECl was present

SUMMARY

1. Triethyl-2-hydroxyethylammonium chloride (TECl) possesses a low chronic oral toxicity as well as low acute oral toxicity; the LD₅₀, about 316 mg. for rats weighing 160 g., indicates that TECl is approximately twice as toxic as choline chloride when given orally.

2. Lipotropic dose-response curves for TECl and choline chloride are presented from which the lipotropic activity of TECl is seen to be about one-fifth of that of choline chloride (on a molecular basis).

3. Male rats did not exhibit any lack of lipotropic response to TECl, i.e. both sexes responded similarly.

4. Supplements of TECl added to hypolipotropic basal diets did not permit growth, but did prevent the development of haemorrhagic kidneys in weanling rats. The significance of this apparent confirmation of statements in the literature is discussed.

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The Effect of Surface-active Agents on Bacterial Glutamic Decarboxylase and Glutaminase

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In previous papers (Krebs, 1948; Hughes, 1949) it was shown that *n*-hexadecyltrimethylammonium bromide (Cetavlon) accelerates the decarboxylation of glutamate and the deamidation of glutamine by intact cells and extracts of *Clostridium welchii*, *Proteus morgani* and *Escherichia coli*. Kinetic studies (Hughes, 1949) suggested that the effect of the detergent might be due to the removal of a competitive inhibitor. In the present work the effect of a variety of surface-active agents on the bacterial glutamic acid decarboxylase and glutaminase has been studied. Acceleration was given only by those cationic surface-active agents which can form micelles, and the concentration of the agents required to form micelles was about the same as that producing maximum acceleration.

MATERIAL AND METHODS

Organisms and extracts. Intact washed cells and extracts of *Cl. welchii*, *Pr. morgani* and *Esch. coli* were prepared as previously described (Hughes, 1949).

Glutamine. Crude L-glutamine (66% pure as judged by amide/N estimation) was prepared from mangel-wurzels by the procedure described by Vickery, Pucher & Clark (1935). It contained, among other impurities, 2% arginine and traces of Zn (see Hughes, 1948), and was freed from these by the following method (see Archibald, 1945). A solution of crude glutamine (35 g.) in 400 ml. water at 60° was cooled to room

temperature, saturated with H₂S, and left for 2 hr. After addition of norite (4 g.) the suspension was filtered through paper on to a column (1.5 cm. diameter and 30 cm. long) of 60–80 mesh permutite (Permutite Co. Ltd., Gunnersbury Avenue, London, W. 4), which had been previously washed with 0.5% NaCl. The glutamine solution was allowed to run through by gravity, and the column was washed twice with 20 ml. water. The combined washings and filtrate were evaporated under reduced pressure at 40° until crystals appeared. After addition of warm ethanol (2 vol., at 40°), the solution was left at room temperature and then at 2° until crystallization had finished. The glutamine crystals were collected, recrystallized from water-ethanol as before, washed with 60% ethanol, 40% water (v/v) mixture and dried *in vacuo* over P₂O₅. Yield 22 g. (Found: amide N, 9.4; total N, 18.9; CO₂ evolved by the method of Gale (1947), 28.6; arginine, 0.01. Calc. for C₆H₁₀O₅N₂: amide N, 9.6; total N, 19.2; CO₂, 29.6%.)

***n*-Alkyltrimethylammonium bromides.** The Cetavlon (*n*-hexadecyltrimethylammonium bromide) was a specially pure sample supplied by Mr S. Ellingworth, Imperial Chemical Industries (Pharmaceuticals) Ltd. Other homologues in this series were synthesized from the *n*-alkyl bromides by treatment with trimethylamine. The alkyl bromides were prepared from the corresponding alcohols which were obtained as follows: commercial samples of *n*-heptyl, *n*-decyl and *n*-dodecyl alcohols were supplied by L. Light and Co. Ltd., Wraysbury, Bucks, and *n*-octadecyl alcohol by British Drug Houses Ltd. These were purified by fractional distillation. Tetradecyl alcohol was prepared by the method of Levene & Taylor (1924) from recrystallized