ε-CAPROLACTAM

BY

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Lactams are internal amides of amino-acids as lactones are internal esters of hydroxy acids. Thus : γ -Hydroxy butyric acid on losing one molecule of water gives γ -butyrolactone

The formation of lactones is an example of a general tendency of carbon chains to form five or more membered rings when the appropriate conditions are created. The lactone is readily hydrolysed to the hydroxy acid.

Similar types of change are brought about by heating γ , δ , ϵ , and ζ amino-acids to the temperature of fusion, thus :

γ -amino butyric acid loses one molecule of water and gives γ -butyrolactam

The δ -lactam of valeric acid is a six-membered ring and the ϵ -lactam of caproic acid is a seven-membered ring.

Cyclic amides are foreign to the animal body although they are readily formed by the condensation of amino-acids, e.g. 2-5-dioxo-piperazine

from glycine $\begin{array}{c} \text{CO-NH} \\ | \quad | \\ \text{CH}_2 \quad \text{CH}_2 \\ | \quad | \\ \text{NH-CO} \end{array}$ by the action of dehydrating agents.

Manufacture of ϵ -Caprolactam and its Polymer

The general method for the preparation of δ , ϵ , and ζ lactams and their parent amino-acids is the formation of the appropriate cyclic ketones from which, by way of the oximes, the cyclic amide is produced by the action of concentrated sulphuric acid. The last stage is known as the Beckmann transformation. Thus, starting from phenols, the intermediate reactions are as follows :

This manner of preparation of the lactam from phenol recalls in its earlier stages the first reactions

in the synthesis of the linear super-polyamide nylon, in which the alkaline oxidation of cyclohexanol or cyclo-hexanone yields the di-basic adipic acid, which on being melted together with hexamethylene diamine gives the polyamide. Since two molecules each with six carbons are involved, the polymer is referred to as "nylon 66".

The "peptide" or amide link --CO--NH-- will be familiar. In the nylon synthesis this link arises from two sources which give rise to the salt. Cross linking at the nitrogen atom also occurs. ϵ -Caprolactam can, by heating its aqueous solution to 260°C . at ordinary pressures, be transformed into a linear polymer thus :

This polymer, from which the synthetic fibres are spun, is a German discovery known as "perlon L". It is unusually pure for a technical product, but is stated to contain normally approximately 1% of the monomer. Perlon L possesses a crystalline structure and in its physico-chemical properties is most readily compared with a metal. Its molecular weight is about 20,000 (Gabler, 1948).

Perlon U is the polymer derived from the condensation product of hexamethylene di-isocyanate and 1-4-butane diol.

Grilon is another polymer envisaged which will involve the condensation of ϵ -caprolactam by heat and pressure.

Working Conditions in the Manufacture of Perlon L

Experience of the manufacture of "perlon L." is greatest in Germany. Hohensee (1951) has given the most recent account of the working conditions in which the polymer is manufactured and the pharmacological properties of ϵ -caprolactam. The batch process is carried out in closed autoclaves which are opened only to charge fresh monomer. At the lower pole of these autoclaves there are the spinneret feeds: the extruded fibres emerge straight into the air, solidify, and are wound on bobbins.

The atmosphere in the spinning rooms was found to contain 61 mg./m.^3 and that of the laboratories $16\text{--}17 \text{ mg./m.}^3$ as lactam (determination by passage of air into strong acid and estimation of ammonia

nitrogen in the Kjeldahl manner). The vapour contamination consists of lactam at various stages of polymerization (dimer and trimer mainly), as well as lactam itself. The vapours condense in the cold air and dust deposits on door lintels, window frames, and beams both as a film and in crystalline form. At the high atmospheric concentrations given above it is not surprising to find that a bitter taste is experienced.

Hohensee (*loc. cit.*) reports that some workmen in the spinning section complained that at the end of the shift they were irritable, nervous, uncontrolled, and in some measure mentally confused. The symptoms were alleged to be aggravated when particularly dusty jobs (e.g. frequent changing of the spinnerets) were undertaken repeatedly during the day, with the occurrence of epistaxis, upper respiratory catarrh, with dry and splitting nose and lips. Other workers complained of flatulence, heartburn, and a heavy feeling in the stomach. All these symptoms disappeared after a period in the fresh air. The epistaxis and respiratory catarrh were attributed by Hohensee to the local irritant action of the lactam dust.

As these were the sole complaints and no other pathological disturbances were reported from the factory, in spite of obviously intense exposure to dust and vapour of monomer and polymers, it was concluded with some assurance that the toxic properties of the materials are not severe.

Properties of ϵ -Caprolactam

ϵ -Caprolactam or cyclohexanone iso-oxime

is a white crystalline solid and is extremely soluble in water. Recrystallized from ether it yields beautiful transparent rhombs with a melting point of 68° to 70°C . The compound tends to take up water in warm, humid air. ϵ -Caprolactam is readily hydrolysed to ϵ -amino caproic acid by heating in dilute mineral acid or alkali. It is stable in aqueous solution at 100°C .

ϵ -Amino caproic acid was prepared in our experiments from the lactam by hydrolysis in N.HCl , removal of the Cl by Ag_2O , precipitation of excess silver as sulphide and recrystallizing from the concentrated, aqueous solution by adding ethanol in which the amino-acid is practically insoluble. It crystallizes from cold alcohol in transparent, flat, hexagonal prisms. Its melting point is 201° to 202°C . It gives the ninhydrin reaction.

Toxicology of ϵ -Caprolactam

In the section on lactams Richter (1934–47) states that “the amino acids are not poisonous, but that the γ and δ lactams are violent, strychnine-like poisons, affecting the spinal cord and producing convulsions; ϵ -caprolactam . . . acts, physiologically, as a nerve poison”. Lactams have weakly acid and weakly basic properties.

The first significant contribution to the toxicology of these compounds seems to have been that by Jacoby, Hayashi, and Szubinski (1903), although Jacoby had already shown in 1901 that the cyclic iso-oximes are powerful convulsants. Jacoby and others, interpreting their physiological results, stated that the effect of introduction of the $+NH$ group into the ring of cyclic ketones was to replace a motor and central paralysis by a stimulant action of the “medullary convulsion centre” and later paralysis in the medulla.

They go on to say that the relatively simple cyclic iso-oximes (lactams) (piperidone, hexanone-iso-oxime, suberone-iso-oxime, and probably also pyrrolidone) produce convulsions in frogs and mice and must be included in the picrotoxin group. The convulsions are not reflex in type (i.e. strychnine-like); they are typically clonic in character and produced, they say, “durch Erregung des in der Medulla gelegenen Krampfzentrums”. ϵ -Caprolactam, then, according to Jacoby and others is quantitatively the least toxic of these iso-oximes or lactams.

The description of the effects of ϵ -caprolactam in mice given by these authors may be summarized thus: the effects can be observed with a dose of 0.5 g./kg. subcutaneously; there is a mild increase in reflexes; intermittent muscle twitches appear and these develop into clonic convulsions; the convulsions are discontinuous and between onsets the animals are “narcotized” (a term used by the authors to indicate maintenance of uncomfortable postures); the animals emit cries; the clonic change over to tonic convulsions and animals die in the latter stage.

Hohensee's results for ϵ -caprolactam gave the L.D.50 values for mice as 0.75 g./kg. (subcutaneous), 0.58 g./kg. (intraperitoneal), 0.48 g./kg. (intravenous), and the L.D.100 was 1.20 g./kg. (by mouth).

These values are of the same order as those obtained by Jacoby and others (*loc. cit.*) and Dieke, Allen, and Richter (1947). Mice sustained without sequelae 42 daily doses of 50 mg./kg. and 150 mg./kg. by mouth and similar doses subcutaneously. Guinea-pigs sustained 26 to 30 daily exposures of five to eight hours to the vapour

of a lactam melt (maximum temperature 180° C.) without any pathological effects. Application of 5% and 10% solution to the shaven skin of guinea-pigs, to the corneae of rabbits, and to the skin of human subjects gave no irritant reaction. In the rabbit 10 mg./kg. intravenously led to an ephemeral rise in blood pressure, and 50 mg./kg. in the cat was also pressor (20 mm. Hg), the effects passing off in 20 to 25 minutes. The pressor effect was not prevented by decapitation or vagotomy or atropine. Morphine inhibition of respiration in the rabbit was reversed by caprolactam, 500 mg./kg. being equivalent to 50 mg./kg. cardiazol.

The conclusions reached by Hohensee (*loc. cit.*) may be paraphrased thus: acute poisoning of human subjects by ϵ -caprolactam is impossible even in unfavourable industrial conditions; chronic absorption may proceed at high levels for extended periods without toxic effects: the low volatility of the compound and very high doses required renders poisoning by inhalation most improbable.

Toxicological Experiments

Nylon 66 is being developed in Great Britain and as the monomer (and lower polymers) is not only used in the manufacture but is present also in the final product, it was necessary to make sure that it was not likely to be a serious hazard in spite of its convulsant properties.

Hohensee's findings are a sufficient indication of the low quantitative toxicity. Our object was not to repeat his experiments, but, among other things, to observe what happens at different dose levels.

The ϵ -caprolactam used in our experiments had a melting point of 68° C. It is extremely soluble in water and ethanol, relatively poorly soluble in ether and the lower glycols, but soluble in chloroform and trichloroethylene to the extent of 1 g./ml.

Effects of Intraperitoneal Injection of ϵ -Caprolactam in Rats

Albino rats were used which belonged to our colony maintained for years in good health and reproductive capacity.

All injections were given in 0.5 ml. distilled water.

Toxic signs begin to appear clearly in many cases at 350 to 400 mg./kg., but recovery is general. At about 500 to 600 mg./kg. these signs are more marked and there are cries, tremors, apprehension, depression of temperature, and sometimes chromodacryorrhoea. But again there is recovery. In general, with our animals, we have not had deaths at doses below 800 mg./kg. Even 800 mg./kg. is not always

a fatal dose (mortality 66%) in spite of the appearance of delayed spasms.

At 900 mg./kg. death is certain, but may be delayed for a considerable time, and at 1,000 mg./kg. death is invariable in a few minutes. Convulsions are, when fully established, epileptiform, violent, and intermittent, and accompanied by salivation and bleeding from the nares. Judging from the condition of the corneal reflex and the fall in temperature and the tendency to lie quiescent between convulsions, there is also central depression.

Disturbances in heat regulation are a regular feature of the action of convulsant poisons. In the case of ϵ -caprolactam the marked fall in temperature when observed is best seen in the periods of depression between convulsive attacks. However, the fall in temperature may occur even in the absence of convulsions, but there is always an accompanying depression. Death was always due to respiratory paralysis, the heart ceasing to beat some minutes later.

If recovery occurred, there were no sequelae whatever the dose.

Intravenous Injection of ϵ -Caprolactam in Rabbits

Six rabbits were injected intravenously with doses ranging from 100 mg./kg. to 300 mg./kg. The effect was that of an intense, transient stimulation of the medulla, in many respects similar to that produced by picrotoxin at doses of 200 mg./kg. and over. There were no deaths.

The signs observed were apprehension, salivation, accelerated respiration, cries, muscular tremors, convulsions, opisthotonus, mydriasis. No fall in rectal temperature was noted. The rapidity of onset and transience in the case of intravenous injection is probably due to a rapid fall from the effective concentration by virtue of rapid removal from the blood or metabolism, or both.

There is thus no doubt that ϵ -caprolactam is a convulsant poison in both the rat and the rabbit.

On the other hand, the toxic properties are lost after the compound is hydrolysed to ϵ -amino caproic acid, even an intraperitoneal dose corresponding to that invariably fatal to rats (1 g./kg.) being totally without effect.

Although Thomas and Goerne (1914) recorded that ϵ -amino caproic acid is not "indifferent", 1.5 g./kg. subcutaneously in a rabbit producing death in 10 hours, no convulsive effects were recorded, and the necropsy of the animal used showed rather dubious changes.

It seems reasonable to conclude that hydrolysis *in vivo* would be an effective mode of detoxication.

Effect of Narcotics on the Convulsions Produced by ϵ -Caprolactam

It is common clinical practice to use intravenous barbiturates to combat convulsions, the danger being, however, that the barbiturate may produce so powerful a depression that it, in turn, becomes a cause of death. The short-acting barbiturates are the agents of choice. Conversely, convulsants, (e.g. picrotoxin, cardiazol) as symptomatic antidotes to narcotics are less effective and dangerously high doses may be required.

In testing the value of a chemical or a symptomatic antidote to a toxic agent, it is usual to administer the antidote after the toxic agent has begun to exert its effect. We used "veronal", the sodium salt of 5.5 di-ethyl barbituric acid (long acting), and tested (a) ϵ -caprolactam after "veronal", (b) ether after ϵ -caprolactam, and (c) "veronal" after ϵ -caprolactam.

Veronal Followed by Lactam.—Veronal is tolerated by rats in very high doses. Thus, we have found 120 mg./kg. intraperitoneally leads to incoordination and drowsiness; 240 mg./kg. produces narcosis; 360 mg./kg. deep narcosis and only 33% deaths. In all there is a considerable fall in temperature. In man the narcotic dose of "veronal" is about 0.3 to 0.5 g. by mouth, and the fatal dose lies between 4 g. and 10 g., i.e. between 60 mg./kg. and 150 mg./kg. approximately.

The inhibition of the action of the lactam by "veronal" is unequivocal as is also that of the "veronal" by the lactam.

Simultaneous intraperitoneal administration of 36 mg./kg. "veronal" and the invariably fatal dose of 1 g./kg. ϵ -caprolactam to three rats led to epileptiform convulsions, cries, chromodacryor-rhoea, loss of corneal reflexes, and bitten tongue, but no deaths. If an interval is allowed to elapse between the barbiturate and the lactam injections, complete absence of all signs of ϵ -caprolactam effects can be obtained.

The dose of "veronal" is naturally a determining factor, for whereas 36 mg./kg. of "veronal" can inhibit the effects of 1 g./kg. of ϵ -caprolactam even after an interval of about two hours, 14 mg./kg. "veronal", effective even after 60 minutes, is ineffective after two hours.

The inhibition of the convulsive action of ϵ -caprolactam by the barbiturate is not more striking than the inhibition of the narcotic effect of the latter by the former. Thus, 1 g./kg. ϵ -caprolactam administered 21 minutes after the narcotic dose of "veronal" (240 mg./kg.) prevented all signs of narcosis, the animal surviving without sequelae.

Ether Narcosis after ϵ -Caprolactam.—If a lethal dose of ϵ -caprolactam be injected intraperitoneally in rats (1 g./kg.), the convulsions which rapidly follow can be controlled by ether inhalation and the animal maintained alive for many hours. If the ether control is stopped the emergence from the narcotic stage is accompanied by convulsions which can be extremely violent. These can be controlled again by ether and the process repeated for many hours. The conclusion seems inescapable that the lactam is maintained at convulsive levels either in the blood or in the central nervous system, and that detoxication is either inhibited or very slow in these conditions.

It would appear, therefore, that narcosis with a volatile anaesthetic like ether must be maintained for a considerable time if the danger from an ordinarily lethal dose of ϵ -caprolactam is to be overcome. Ether is less effective than "veronal" in combating the convulsive action of ϵ -caprolactam.

ϵ -Caprolactam Followed by Veronal.—Since the invariably fatal dose of lactam in rats of 1 g./kg. intraperitoneally produces death in a very few minutes, little time is available to take antidotal measures. A test dose of 0.8 g./kg. ϵ -caprolactam was therefore taken which, while producing severe symptoms, does not kill all animals. Two groups of six rats each were taken. One group received 0.8 g./kg. ϵ -caprolactam intraperitoneally, and the other group the same dose followed by an intraperitoneal injection of "veronal" (36 mg./kg.) as soon as severe toxic signs appeared.

Severe signs appeared in both groups, but the mortalities were respectively 4/6 and 1/6.

A marked feature in the groups given "veronal" as well as ϵ -caprolactam was bleeding from the nares, even when convulsive movements were slight.

Long-term Administration of ϵ -Caprolactam to Rats

Although the gross acute toxicity of the compound is manifestly low, it was considered desirable to carry out a long-term experiment using the growth rate of rats as an index of insidious effects.

Two groups, each of six rats, were taken from two litters of the same age and, after a few days' maintenance in separate cages to accustom the animals to solitary life, one (Group E) had its water supply replaced by an aqueous solution of ϵ -caprolactam, 0.75 g. per 100 ml. The other (Group C) was the control group.

This concentration had been found in preliminary trials to be the highest the rats would accept.

The solid part of the diet was the same for both groups and was identical with that used for years in

this laboratory, providing excellent growth and health. Fluid intake was measured daily. Weights of the animals were determined at frequent intervals. The experiment continued for 118 days. The average weights of both groups were identical at the onset of the experiment at 124 g. Table 1 summarizes the findings during the first 70 days of the experiment.

TABLE 1

—	Group C (6 rats)	Group E (6 rats)
Initial average weight	124 g.	124 g.
Average weight after 70 days	319 g.	287 g.
Total fluid intake per kg. body weight	10.33 litres	6.22 litres
Total lactam ingested per kg. body weight	0	46.65 g.
Average lactam ingested per kg. per day	0	0.667 g.

At this stage the lactam was withdrawn, with the result that in the succeeding 20 days Group E reached an average weight of 328 g. while Group C remained at 320 g.

In the succeeding 29 days Group C was put on the lactam solution, while Group E continued on tap water. At the end of this period the average weights were Group C 336 g., and Group E 356 g.

It thus appears that the depression in growth seen in Table 2, and attributable to the great diminution in fluid intake, is completely reversible. During the whole period of experiment neither group of animals showed any signs of ill-health. It is concluded, therefore, that growing rats will continue to ingest (and, we assume, absorb) 0.667 g./kg./day for long periods with no other sign of disturbance than a depression of the rate of growth.

Effect of ϵ -Caprolactam on Blood Pressure and Respiration

Blood Pressure.—According to Hohensee (*loc. cit.*) 10 mg./kg. ϵ -caprolactam produces an ephemeral rise in blood pressure in rabbits. In cats, 50 mg./kg. produces a rise in blood pressure of 20 mm. Hg, and the rise is said to have been maintained for 20 to 25 minutes. This rise occurred also in decapitated, vagotomized, and atropinized cats.

However, there is sometimes shortly after the injection an initial fall in blood pressure. On the isolated frog's heart, there is a passing inhibition at $\frac{1}{10^4}$ to $\frac{1}{5 \times 10^3}$ but later there is a positive inotropic effect. Hence Hohensee suggests that there may be enough cardiac inhibition in the mammal to

produce the passing depressor effect and thereafter the rise will occur as a result of the stimulus to the force of the heart.

Our experiments were designed simply to see if any significant change in blood pressure could be produced by intravenous injection.

Fig. 1 shows the effect of moderate to large single doses of ϵ -caprolactam in the rabbit. Five repeated doses of 23 mg./kg. were required to produce a steady rise in blood pressure from 82 to 100 mm. Hg, a fifth dose no longer producing any effect, but in the following 20 minutes the pressure fell to 50 mm. At this point an injection of 230 mg./kg. led to a sharp but temporary fall to 34 mm., with an accompanying rise in respiratory rate. The conditions *quo ante* were re-established some four minutes after this injection.

The cat (Fig. 2) responded invariably to 136 mg./kg. with a fall in blood pressure and a significant rise in respirations. Two doses brought the blood pressure down from an initial 102 mm. to 46 mm., and the respirations up from 28 per minute to 40 per minute. Atropinization (5 mg./kg. subcutaneously) did not affect respiration, but the subsequent injection of 136 mg./kg. ϵ -caprolactam increased

Fig. 2.—Effect of ϵ -caprolactam on blood pressure and respiratory rate of cat.

the respiratory rate over threefold. Convulsions appeared and were most violent, lifting the animal from the table. The convulsions appeared after the great rise in respiratory rate, and did not increase the rate to any marked extent. Vagotomy does not prevent the depressor or the respiratory stimulant effect.

In the next experiment it will be seen that respiration is accelerated in the total absence of convulsions.

Fig. 1.—Effect of ϵ -caprolactam on blood pressure and respiratory rate of rabbit.

Respiration.—Although we have seen the intense respiratory stimulation induced by ϵ -caprolactam in the anaesthetized, atropinized cat, it was felt desirable to repeat the test in the unanaesthetized animal in which the respiratory centre had been markedly depressed.

Fig. 3 shows the effect of various subcutaneous doses of ϵ -caprolactam on the slowed respiratory rate of rabbits injected subcutaneously with 5 mg./kg. morphine hydrochloride.

The doses of lactam varied from 100 mg./kg. to 500 mg./kg. and the response was clear in all cases.

The sensitivity of the respiratory centres varied, the quantitative response not corresponding to the size of dose.

Fig. 3.—Effect of subcutaneous ϵ -caprolactam on respiratory rate of morphinized rabbits.

The maximum stimulation of the centre occurred at about 20 minutes (14–31) after the lactam injection, at which time probably the highest blood lactam concentration had been reached.

Slight to severe tremors were produced by all the doses of lactam but no convulsions.

Inhalation of ϵ -Caprolactam Dust

Hohensee (*loc. cit.*) points out that the low volatility of this compound renders poisoning by this route most improbable, and substantially impossible. He exposed guinea-pigs to air which had been passed over a lactam melt at 180° C. (concentration in atmosphere 51 mg./m.³). Exposure for five to eight hours per day for 26 to 30 days resulted in no pathological effects nor any evidence of increased or abnormal mobility. Slight evidence of irritation of the nasal mucosa was noted.

In our experiments, three guinea-pigs were exposed to concentrations of very fine particulate dust produced by Gage's method (to be published), whereby very fine sprays of solutions can be delivered into a chamber so that the solvent evaporates and the fine dust concentrations are set up dynamically. The vast majority of the particles formed from the aqueous spray were below 5 μ in size. The concentrations of ϵ -caprolactam (determined analytically) were varied on different days from 118 mg./m.³ to 261 mg./m.³. The animals were exposed for seven hours daily for seven days. Except for occasional coughing, no adverse effects were noted.

The total amounts of the compound absorbed in our experiment and in that of Hohensee were clearly of the same order, and we draw the same conclusions.

Application of ϵ -Caprolactam to the Skin of Normal Human Subjects

It will be recalled that Hohensee (*loc. cit.*) applied 5% and 10% solutions of ϵ -caprolactam to the skin of guinea-pigs and of human subjects, as well as to the corneae of rabbits without finding evidence of irritant properties. Application of the 5% solution to the skin of one man and two women for 35 days of eight hours each led to no evidence of irritation. One of the women, who was pregnant (six months), showed a mild erythema after 20 days' application, and by the thirtieth day the experiment had to be stopped. The other two subjects showed no adverse effects whatever. The cause of the erythema in the pregnant woman was not clarified, but

Hohensee evidently did not attach much importance to it.

In our experiments we applied 5% aqueous ϵ -caprolactam to the skin of the inner forearms of six normal persons (one fair girl, one dark girl, two fair men, two dark men) as a 4 cm. $\times$ 3 cm. patch test left in contact for 48 hours. There were no reactions produced by the ϵ -caprolactam.

In a second series of tests a 5% alcoholic solution of ϵ -caprolactam was applied by placing six drops on the same area: the alcohol was allowed to evaporate and the residue kept undisturbed in contact with the skin throughout the day. The process was repeated twice daily for four days. No irritant effects of any kind were produced.

Similar experiments were carried out with a 5% olive oil solution of ϵ -caprolactam, and again it was found with five subjects that no irritant effects were produced during four days of application twice a day.

Excretion and Metabolism of ϵ -Caprolactam

With so soluble a compound and so low a quantitative toxicity it was to be expected that excretion or detoxication (or both) would be rapid.

A simple process of detoxication would be to hydrolyze it, thus opening the ring and yielding the ϵ -amino acid. The ultimate disposal of this amino-acid would depend upon whether the organism could metabolize it.

In respect of the possible metabolism of this amino-acid, reference may be made first to the work

Rat No.	Normal Daily Amino-N Excretion (mg. per 100 ml. urine)									Mean Value	Standard Error of Mean
1	4.3	12.3	8.5	7.7	7.7	—	—	—	—	8.1	1.43
2	8.6	10.2	8.9	10.2	7.7	6.6	7.5	7.1	10.0	8.6	0.46
3	9.6	10.0	9.6	8.3	8.1	7.2	10.0	7.1	12.5	9.1	0.56
4	8.6	10.2	8.9	10.2	7.7	6.6	7.5	7.1	10.0	7.7	0.55
5	9.6	10.0	9.6	8.3	8.1	7.2	10.0	7.1	12.5	8.2	0.66
Grand mean										8.4	0.35

by this method from 0.1% aqueous solution was 73%, and from 0.1% in normal urine was 71%.

Determination of Lactam in Faeces

In experiments on the excretion of lactam it was necessary to determine possible excretion in the faeces.

The following was the method used :

The day's faeces were weighed, and 5 g. (or total faeces if less than 5 g.) was ground up with 15 g. anhydrous Na_2SO_4 . The mass was extracted twice with chloroform at room temperature. The combined deep-green extracts were freed from chloroform by distillation in a boiling water bath. Some 20 ml. of distilled water was now added to the green extract and the flask replaced in the boiling water bath to remove traces of chloroform. When the volume has thus been reduced by about half, it is found that the highly pigmented matter remains adherent to the sides of the flask and the water extract is clear. The aqueous extract is now filtered into a flask, several washings combined and then hydrolyzed (2N HCl) at 100° C. for one and a half to two hours, and the resulting amino-acid determined titrimetrically.

Applying this method to rat faeces containing 50 mg. per 5 g. faeces, a yield of just over 72% is readily obtained. Appropriate correction was therefore made in individual determinations.

Excretion of Lactam and Amino-acid after Intraperitoneal Injection of Lactam

In the following experiments the excretion of lactam and amino-acid was determined after intraperitoneal injection of lactam in the quantities stated. The determination in urine continued in each case until no lactam could be detected in the urine.

No certain conclusion can be drawn from our figures (in Table 3). Tentatively we may say that circumstances may exist in the rat when the metabolism of ϵ -amino caproic acid appears difficult, and in such circumstances injected lactam can be largely accounted for by excreted lactam and amino-acid. The problem will be pursued in further experiments by the administration of ϵ -amino caproic acid, but we may refer again to Dakin (*loc. cit.*), whose results indicated that the metabolism of the natural di-amino caproic acid is not straightforward, and to Corley (*loc. cit.*).

Excretion of Lactam and Amino-acids by Rabbits after Intraperitoneal Injection of ϵ -Caprolactam

In similar experiments with three rabbits, 400 mg./kg. ϵ -caprolactam were injected intraperitoneally and analyses of urine and faeces continued for four days. The percentages of the dose appearing in the urine and faeces as lactam were 8.7, 22.3, 10.1,

TABLE 3
EXCRETION OF LACTAM AND AMINO-ACIDS BY RATS
AFTER INTRAPERITONEAL INJECTION OF
 ϵ -CAPROLACTAM

Lactam Injected (mg. N.)	Lactam in Urine (mg. N.)	Amino-acid* Excess in Urine (mg. N.)	% of Dose Accounted for in Urine in Periods of 2 to 5 Days		
			As Lactam	As Amino- acid	Total
12.3	8	None detected	65	?	—
12.3	4	Not deter- mined	32.5	?	—
12.3	4.5	Not deter- mined	36.5	?	—
14.8	6.9	5.2	46.6	35.3	82
14.8	6.9	8.3	46.6	53.4	103
16.0	3.5	7.9	21.9	49.1	71
17.3	2.9	10.4	16.8	60.2	77
(Control values for amino-acid excretion determined during 9 days before injection)					
14.8	1.4	Dubious	10	?	—
14.8	3.4	8.9	22.8	60	83

Control values for amino-acid excretion determined during two to three days before injection.

and no additional amino-acid N could be detected in the urine.

It would thus appear that the rabbit can metabolize ϵ -caprolactam almost completely. Of the 2.24 g. lactam injected into these animals, 86% was not accountable for either as lactam or amino-acid. This finding agrees with that of Thomas and Goerne (*loc. cit.*).

We have not had an opportunity to study the utilization of ϵ -caprolactam in human subjects: this will perhaps be possible later when it is manufactured.

Discussion

The practical question is whether, in view of the experimental evidence, the assurance can be given that the hazard to operatives who might be exposed to considerable concentrations of the monomer (ϵ -caprolactam) is negligible. Hohensee (*loc. cit.*) gives this assurance on the grounds that the proportional acute fatal dose in man would be about 70 g., that chronic administration of high doses in animals is well tolerated, and that the low volatility of the compound renders toxic effects most improbable by inhalation. There is also the additional evidence that men have worked in high concentrations with only minor complaints, and that there is no evidence of direct skin irritation in human subjects. (Hypersensitivity may be found in some cases.) From a variety of pharmacological observations made by Hohensee (inhibition of the isolated gut,

inhibition of the frog's heart), and from our own demonstration of depressive action and ultimately respiratory paralysis, we must conclude that ϵ -caprolactam is a protoplasmic poison as well as a convulsant.

These effects are produced only by relatively large doses. It is the business of those responsible for industrial health to preclude the absorption of any such material, even if there is little chance of cumulation. Cumulation, indeed, is ruled out by the long-continued ingestion of the compound by our rats (0.67 g./kg./day).

The symptoms complained of by men exposed to high concentrations of monomer dust were irritability, nervousness, loss of control, and some confusion (Hohensee, *loc. cit.*). Such a consistent group of symptoms cannot be ignored. It is in close correspondence with what would be expected from the reaction of animals to sub-lethal doses.

It is too facile to suggest, as Hohensee appears to do, that most of these complaints were psychological or due to the odour and taste of the compound. The fact that the German workers only complained of those symptoms when exposed to a high concentration is in itself significant.

We take the view, therefore, that in spite of the quantitatively relatively low toxicity of ϵ -caprolactam, the possible effects of the exposure of workers to high atmospheric concentrations of the vapour or dust constitute a potential hazard. This view is taken because doses far below lethal in animals can continue to cause mild tremors, increased reflex activity, and apprehensiveness for many hours.

Summary and Conclusions

ϵ -Caprolactam is a convulsant poison in rats, rabbits, and cats.

Quantitative toxicity is very low by all routes. Pharmacological effects are, however, evoked in the intact animal by doses of about 100 mg./kg. and over.

It can be tolerated orally in the drinking fluid in large doses without toxic symptoms by growing rats for long periods. Because of its unpleasant taste, the growing animals voluntarily ingest only sufficient of the solution to assure growth, but at a

level much below normal. This effect is completely and swiftly reversible.

ϵ -Caprolactam can prevent the narcosis induced by "veronal", and "veronal" can inhibit or stop the convulsions due to caprolactam.

In large doses it is a powerful respiratory stimulant and mild circulatory depressant. In smaller doses a slight pressor effect on the circulation can be produced.

ϵ -Caprolactam is not a skin irritant and no indication has been found that it could act as a sensitizing and dermatitic agent.

High concentrations of fine caprolactam dust (118 to 261 mg./m.³) can be respired by animals for long periods without manifest adverse effects.

ϵ -Caprolactam is excreted by rats partly as lactam and partly as ϵ -amino-acid, and sometimes appears to be almost wholly accounted for in this way. It appears, therefore, that the ϵ -amino-acid may not be easily metabolized by the rat.

The rabbit, on the other hand, appears able to metabolize ϵ -caprolactam completely, a small proportion of unmetabolized lactam appearing in the urine after intraperitoneal injection of large doses. It is desirable to discover how the lactam is metabolized in man.

Whilst the likelihood of serious dangers to health from ϵ -caprolactam in the processes of manufacture and use is small, it is considered desirable to preclude the possibility of high concentrations in the working environment and to keep medical records of exposed men, especially concerning the circulatory, respiratory, and nervous systems.

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REFERENCES

- Corley, R. C. (1929). *J. biol. Chem.*, **81**, 545.
- Dakin, H. D. (1922). *Oxidations and Reductions in the Animal Body*. 2nd ed. Longmans, Green & Co, London.
- Dieke, S. H., Allen, G. S., and Richter, C. P. (1947). *J. Pharmacol.* **90**, 260.
- Gabler, R. (1948). *Naturwissenschaften*, **35**, 284.
- Hohensee, F. (1951). *Faserforsch. und Textiltech.*, **2**, 299.
- Jacobi, C., Hayashi, and Szubinski (1903). *Arch. exp. Path. Pharmacol.*, **50**, 199.
- Richter, V. von (1934-47). *Organic Chemistry*, 3rd Eng. ed., vol. 1, p. 450. Kegan Paul, London, and Elsevier Publishing Co., N.Y. Translated from 12th German Edition.
- Thomas, K., and Goerne, M. H. G. (1914). *Z. physio. Chem.*, **92**, 163.