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THE PHARMACOLOGICAL EFFECTS OF MASSIVE DOSES OF NICOTINAMIDE

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Nicotinic acid and its derivatives are natural components of foodstuffs and of a number of intracellular enzymes. They are not very toxic and in the usual therapeutic doses produce no significant pharmacological effects. The transient nitrite-like vasodilatation caused by nicotinic acid is well known (Smith, Ruffen, and Smith, 1937; Robertson, 1941). Since nicotinamide does not produce this side-effect it is preferred for therapeutic purposes. However, on injection of massive doses of nicotinamide we observed certain characteristic phenomena which are described in this paper.

METHODS

Cats, dogs, and rabbits were anaesthetized with pentobarbitone sodium (35 mg./kg. intraperitoneally), if necessary after ether, but ether induction was not used in those experiments in which blood sugar determinations were done. Respiratory movements were recorded by connecting a tambour to a T-shaped tracheal cannula. Carotid arterial pressure was recorded with a mercury manometer. For glucose determinations blood samples in these animals were withdrawn from the femoral arteries and analysed by the method of Hagedorn-Jensen. Urine was collected by insertion of ureteral cannulae in dogs and by vesical cannulae in cats. When rabbits were used without anaesthesia, blood samples were taken from the ear vein for sugar determination and from the heart for estimation of nicotinamide.

For the diuresis experiments groups of four rats of 150–250 g. were fasted for 16 hours, but were allowed water until the start of the experiment.

Nicotinamide was determined colorimetrically as the ferric chloride complex of nicotinohydroxamic acid (Bergmann, 1952). This method is specific for the amide and those of its derivatives which still contain the amide group, e.g., the N^1-methyl derivative cannot be distinguished from nicotinamide, but nicotinic *acid* gives a negative response in the test. When nicotinamide is added to whole blood, deproteinization with 10% trichloroacetic acid (Procedure 1) gives a colorimetric value of 45 Klett units (KU)/μmole/ml. This method has the disadvantage that trichloroacetic acid itself reacts slowly with alkaline hydroxylamine, so that a blank without blood must be run simultaneously in every experiment.

Deproteinization with the Hagedorn-Jensen reagent (Procedure 2) leads to a value of about 55 KU/μmole/ml. The reason for the discrepancy between the two methods is not yet clear and is being studied further. In both procedures the linear relationship between concentration of nicotinamide and extinction has been established. Blood usually gives a blank reading of 240 KU/ml., and urine of 100–150. Occasionally, however, we observed blank readings for urine of 300 KU/ml. and higher. The nature of the chromogenic material in urine is now under investigation, since it is improbable that such large normal blank values are due to the excretion of nicotinamide derivatives.

Procedure 1: 0.5 ml. whole blood is added to 1 ml. of 10% trichloroacetic acid. After standing at room temperature for 15 minutes the tube is centrifuged, and an aliquot of the supernatant (usually 0.75 ml.) is pipetted off. Water is added to give 1 ml. and then 2 ml. of the alkaline hydroxylamine reagent (made by mixing 50 ml. of 2 N hydroxylamine sulphate and 55 ml. of 3.5 N NaOH). The test-tube is left in a water bath at 26° C. for 12–16 hours. Then 1 ml. of 3.5 N HCl is added, the mixture swirled well, and the coloured complex formed by addition of 1 ml. of 0.74 M $FeCl_3$ dissolved in 0.1 N HCl. Readings are taken within 10 minutes. If the reading exceeds 300 KU the solution is diluted with 0.37 M $FeCl_3$ dissolved in 0.1 N HCl.

Procedure 2: 0.5 ml. whole blood is added to 2.5 ml. of a mixture prepared from 5 volumes of 0.45% $ZnSO_4.7H_2O$ and one volume of 0.1 N NaOH. The test-tube is placed in a boiling-water bath for four minutes, then centrifuged, and 1 ml. of the supernatant is added to 2 ml. of alkaline hydroxylamine reagent (prepared from *equal* volumes of the two components). The mixture is treated further as above.

Nicotinamide in urine is determined directly on 1 ml. or a fraction of it. The hydroxylamine solution is made up as in Procedure 2. Urine from the pre-experimental period is used as blank.

Results

Toxicity of Nicotinamide.—The LD50 was determined graphically from the dose-response curve, 20 animals being used for each dose. We find for mice values similar to those obtained by earlier investigators; LD50 after intravenous injection 1.62 g./kg.; LD50 after intraperitoneal injection 1.80 g./kg. The closeness of these two values and of that for the subcutaneous LD50, found by Brazda and Coulson (1946) to be 1.68 g./kg., indicates that nicotinamide is rapidly absorbed into the circulating blood. This conclusion finds support in the experiments reported below. Death usually occurs within 6–12 hours. After large doses the animals first show pronounced tachypnoea, and later prostration and shallow respiration. The toxicity is not altered by intraperitoneal administration of 1.0 g./kg. *d, l*-methionine one hour before the injection of nicotinamide, although death is somewhat delayed.

The toxicity of nicotinamide is much higher for hypophysectomized rats, which succumb to doses of 0.7–0.8 g./kg. intraperitoneally.

Effect on Blood Pressure, Respiration, and Heart Rate.—For these experiments 9 dogs, 12 cats, and 4 rabbits were used. Intravenous injection of 0.5–1.0 g./kg. of nicotinamide produces a sudden fall of blood pressure which reaches a minimum within less than 1 minute (Fig. 1). Then gradual recovery begins; it usually takes 10–20 minutes before a constant level is reached again. In cats little change of heart rate is observed, whereas dogs react with a marked tachycardia (e.g. from 168 to 240/min.). The respiratory movements may stop for the first few seconds, to continue with increased depth and sometimes with increased frequency, so that the pulmonary ventilation is always increased. These effects are also observed after cutting both vagi. The spinal cat shows a corresponding, though less pronounced, fall of blood pressure. The blood pressure and respiratory effects are also obtained after intraperitoneal injection of 0.5–1.0 g./kg. nicotinamide, although they are less abrupt. This shows that the substance is absorbed quickly from the peritoneal cavity, an observation which agrees with those mentioned above in which the LD50 i.p. was found to be very close to the LD50 i.v.

If the blood pressure is raised by intravenous injection of *l*-ephedrine (0.8 mg./kg.), subsequent injection of nicotinamide causes a pronounced fall of blood pressure. *l*-Epinephrine (4 μg./kg.) causes a temporary rise when injected into a cat at the time when the blood pressure has reached its minimum after administration of nicotinamide.

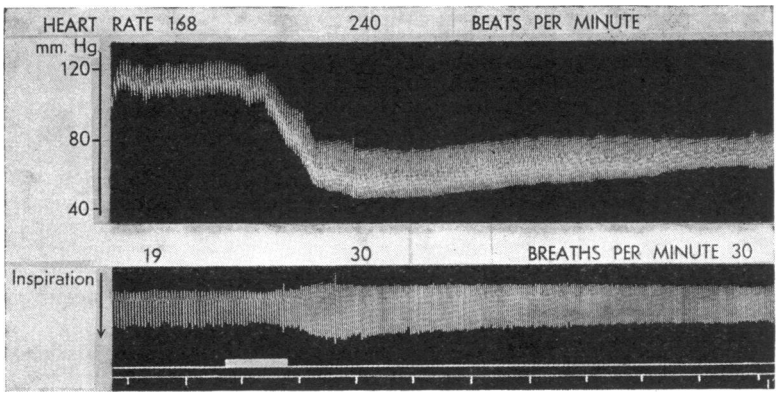

FIG. 1.—Male dog, 14 kg., pentobarbitone. At signal 1 g./kg. of nicotinamide dissolved in 30 ml. saline injected intravenously. Tracings from above downwards: carotid blood pressure; respiratory movements; signal; time in minutes.

The extended recovery period after intravenous injection of nicotinamide may be related to the time required for clearance of the drug from the circulating blood. The results of periodical determinations (Fig. 2) show an exponential decrease of the blood concentration after intravenous injection. A few minutes after injection into dogs, cats, and rabbits only about 20–30% of the total amount is recoverable from the blood. This calculation is based on the assumption that the blood volume is 7% of the total body weight. Thus when 0.5 g./kg. nicotinamide is injected intravenously, these 500 mg. = 4,170 μmoles are distributed within 70 ml. of blood, and we should find immediately after injection a concentration of 59.6 μmoles/ml. The experimental value of 13 μmoles/ml. thus represents about 22% of the calculated maximum. Hence it can be concluded that nicotinamide is distributed very rapidly throughout the extracellular fluid. However, 3–4 hours pass by before

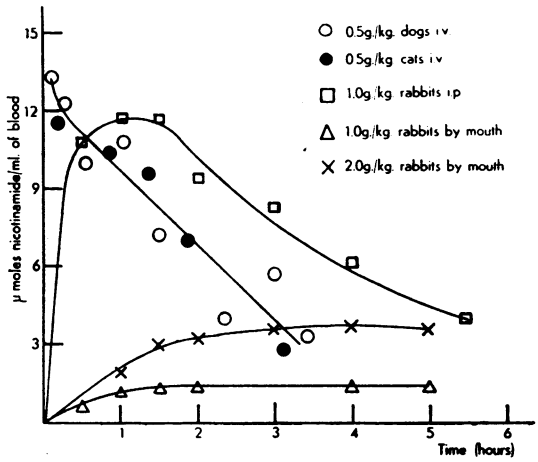

FIG. 2.—Blood concentration of nicotinamide after different routes of administration.

the blood concentration sinks below the level which can be detected by our analytical method. Nicotinamide can be removed from the blood either through the kidneys or into the intracellular space. Since the first possibility is eliminated by the experiments described below, it becomes clear that absorption of nicotinamide by the tissue cells is a rather slow process and probably dependent on an enzymatic reaction.

After intraperitoneal injection (Fig. 2), the blood concentration rises fast, and remains for about one hour at an almost constant level, while absorption into the blood stream and disappearance from the circulation proceed at the same rate. After oral administration a constant blood level of nicotinamide is maintained for several hours, since the speed of intestinal absorption regulates the passage of nicotinamide into the blood (Fig. 2).

TABLE I

THE HYPERGLYCAEMIC EFFECT OF NICOTINAMIDE ON FASTING ANIMALS

Mean of	2 Rabbits	2 Rabbits	2 Rabbits	4 Rats	4 Rats
Dose g./kg.	0·75 i.v.	1·0 i.p.	2·0 by mouth	1·0 i.p.	0·75 s.c.
Min. After Administration	mg. Glucose/100 ml. of Blood				
0	124	120	94	79	95
5	128	—	—	—	—
30	168	132	135	132	106
60	203	153	138	139	121
90	214	173	156	159	132
120	209	195	229	177	148
180	182	213	174	173	120
240	148	204	174	131	101
300	134	177	174	—	96

Effect on Blood Sugar.—Nicotinamide has been shown to protect rats against alloxan diabetes (Lazarow, Liambies, and Tausch, 1950), although the mechanism of this effect is not clear. It was thus surprising to find that nicotinamide produces a large hyperglycaemia, which reaches its maximum after 1–1½ hours, but is still pronounced after 3–4 hours (Table I). After intraperitoneal injection and oral administration a similar effect is obtained both in normal and anaesthetized animals. It is noteworthy that after injection of nicotinamide histological examination of the dog's liver revealed degenerative changes with vacuolization both in the peripheral and central parts of the lobules.

Oliguria.—When we tried to examine the excretion of nicotinamide in the urine of our experimental animals, we observed a considerable decrease in urine flow. In Fig. 3 is shown the effect of intraperitoneal injection of nicotinamide into rats which had been given 50 ml. water/kg. by stomach tube. At a dosage of 1 g./kg. of nicotinamide, urine excretion is almost completely suppressed, while smaller quantities, 0.5 g./kg. and above, cause oliguria, though the total excretion of water finally approaches that of the controls. The effect is approximately proportional to dosage. Intraperitoneal injection of nicotinamide (1 g./kg.) is less effective when the water is also given intraperitoneally than when given by mouth. This may be due to dilution of nicotinamide. A still smaller

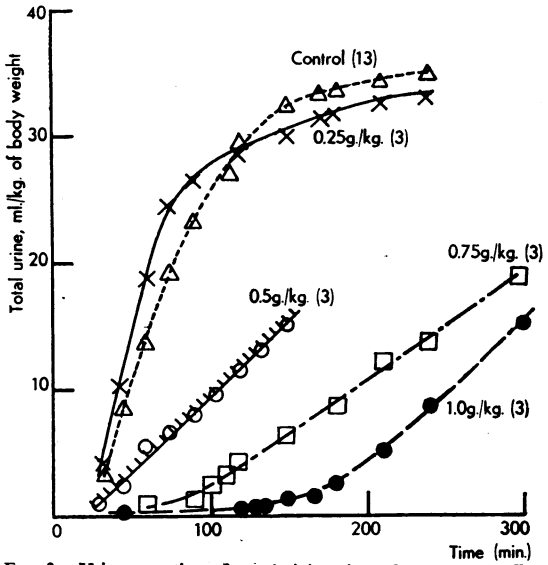

FIG. 3.—Urine excretion after administration of 50 ml. water/kg. by stomach tube and simultaneous i.p. injection of varying doses of nicotinamide. Mean figures from groups of four rats each. Number of groups in brackets.

oliguria is observed when both nicotinamide and water are given by mouth.

If 2.5% saline is given instead of water, the rate of urine excretion is increased and the total amount excreted during the experiment is larger than the saline intake. Nicotinamide reduces the rate of saline excretion, indicating that it does not act through stimulation of the posterior lobe of the hypophysis (see Discussion). In accordance with these findings, the oliguria caused by nicotinamide in hypophysectomized rats (Table II) was very

TABLE II

INFLUENCE OF NICOTINAMIDE ON THE EXCRETION OF URINE IN HYPOPHYSECTOMIZED RATS

Dose g./kg.	No. of Rats	Total Urine Excretion in ml./kg. After (Minutes)									
		30	60	90	120	150	180	210	240	300	360
0	2	4·0	—	5·4	11·2	—	—	—	—	17·4	—
0	4	2·15	—	6·2	—	10·0	—	12·2	—	16·0	—
0	4	5·0	7·0	9·4	10·8	13·7	—	18·1	18·1	—	—
0·4	2	—	1·6	3·0	5·4	—	—	—	—	9·4	—
0·5	4	—	—	0·8	3·5	5·7	7·5	9·7	11·4	—	—
0·6	4	—	—	—	—	—	2·2	—	—	4·05	7·0

Groups of rats received 50 ml. of sterilized water/kg. of body weight intraperitoneally and varying doses of nicotinamide subcutaneously.

great, although it proved impossible to use the same dosage as in normal animals owing to the greater toxicity of the drug in the operated rats.

Finally the effect of nicotinamide on mercurial diuresis was studied. We produced a suitable diuresis in normal rats by injecting the mercurial 2–2½ hours before giving water. Nicotinamide was again very effective in producing oliguria under these conditions, as after administration of saline.

On histological examination of the kidneys the glomeruli appeared normal. The tubular epithelium was swollen and there was hydropic degeneration of the cells lining the collecting tubules. In some places the swelling had led to an obstruction of the tubular lumen; the interstitial tissue showed oedema.

TABLE III

EXCRETION OF NICOTINAMIDE (OR DERIVATIVE)

On the first day groups of four rats received 50 ml. water/kg. body wt. by stomach tube and varying doses of nicotinamide by intraperitoneal injection; water was allowed after six hours. On the second day 50 ml. water/kg. body wt. was given by stomach tube, and water was allowed after four hours. Both urine volume and nicotinamide excretion are calculated per kg. body wt.

Dose of nicotinamide:	0·75 g./kg.		1·0 g./kg.	
Time After Injection in Hours	Urine ml.	Urine mg.NA	Urine ml.	Urine mg.NA
1st day 2	2·4	12·3	—	—
3	0·5	5·5	3·3	30·0
4	1·1	28·0	1·9	31·2
5	4·4	17·7	5·9	55·0
6	2·9	4·0	4·5	34·5
24	19·7	109·0	22·6	240·0
2nd day 1	6·3	13·8	5·1	15·4
2	22·2	7·9	18·4	23·6
3	11·7	1·0	4·1	1·0
4	4·1	1·0	0·6	0
24	16·2	30·6	—	—
	Total (recovered)	230·8mg. (32·5%)		430·7mg. (43%)

Fate of Nicotinamide in the Body.—About 12 hours after administration of nicotinamide the urine excretion of rats becomes normal and remains so. During this period the urine contains up to 10 mg./ml. of nicotinamide (or a derivative of it) depending on dosage. The total amount recovered during 72 hours varies between 25 and 50%, as shown in Table III.

DISCUSSION

The oliguria which follows administration of nicotinamide could be due to a number of causes. The fall in blood pressure could lead to a reduction of renal blood flow and thus of glomerular filtration. There is, however, no correlation between the duration of the hypotension and of the oliguria, and the blood pressure fall was never sufficient to account for complete anuria. Moreover, if this explanation were correct, the return of the blood pressure to normal should be followed by a large urine flow (Rydin and Verney, 1938). In our experiments the rate of urine production lags considerably behind the normal for a number of hours. An indirect action of nicotinamide via the hypophysis has also been excluded. Additional support for this comes from the experiments with saline diuresis, since Brunn (1920) and Gibbs and Fulghum (1946) have shown that the antidiuretic hormone is less effective during a saline diuresis. The only factor to which urine production can be clearly related is the blood concentration of nicotinamide. When the latter falls below a certain level, urine flow starts again, although the excretion of the drug or its metabolites extends over two to three days. It thus becomes probable that nicotinamide acts directly on the kidney in proportion to its blood level by increasing tubular reabsorption. This conclusion is supported by the effect of nicotinamide on saline and mercurial diuresis and by the histological changes observed in the kidneys. It is apparent that nicotinamide is reabsorbed from the glomerular filtrate within wide limits and thus also influences the reabsorption of water. This suggests that the substance actually excreted in the urine is not nicotinamide but a metabolite (see Beyer, Russo, Gass, Wilhoyte, and Pitt, 1950).

The hyperglycaemic action of nicotinamide is another expression of the metabolic changes induced by this substance. It was first thought

that the liver cells are damaged by the high doses of nicotinamide and that the stored glycogen is then converted into glucose. However, analysis of the glycogen content of the liver did not give consistent results. Another possibility is that nicotinamide interferes with the utilization of glucose by the cells. This hypothesis is based on the inhibitory effect of nicotinamide on those enzymes which contain nicotinamide in their prosthetic group (Feigelson, Williams, and Elvehjem, 1951). Nicotinamide produces a suppression of endogenous respiration and could well be responsible for the diminished utilization of glucose. The fact that Lazarow and co-workers (1950) did not observe the hyperglycaemic effect of nicotinamide in rats, although they injected 0.9 g./kg. intravenously, is probably due to differences in experimental procedure (the first blood sample was taken by them after three hours and all samples were pooled).

Summary

1. A method for the determination of nicotinamide in biological fluids has been developed.

2. The toxicity of nicotinamide does not vary greatly with the route of injection, but is lower after oral administration.

3. Massive doses of nicotinamide (0.5–1.0 g./kg.) injected intravenously or intraperitoneally cause a fall in blood pressure and a rise in the rate and amplitude of breathing and sometimes in the pulse rate.

4. Nicotinamide injected intravenously is distributed very rapidly throughout the extracellular space, but absorption by the tissue cells is a slow and prolonged process.

5. Nicotinamide produces a marked hyperglycaemia.

6. Nicotinamide produces oliguria preceded, after large doses, by a period of anuria. This effect is not mediated by the pituitary but appears to be due to a direct action on the tubular mechanism.

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