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DISTRIBUTION OF [^{14}C]BEMEGRIDE IN TISSUES AFTER INTRAVENOUS INJECTION

BY

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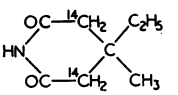
The distribution and excretion of bemegride labelled at the α carbon atoms with ^{14}C were studied in mice, rats, and guinea-pigs. These studies were supplemented by others made by colorimetric and absorptiometric methods on unlabelled bemegride in the body fluids of rabbits and dogs. Bemegride passed rapidly from plasma to all tissues, including brain, muscle, and fat. In addition it passed readily into the cerebrospinal fluid and the aqueous humour and to the foetus. It persisted in the tissues for longer than 24 hr., to some extent preferentially in the central nervous system. Approximately two-thirds of the dose was excreted in the bile, faeces, and urine within 24 hr. The glutarimide ring of bemegride was not completely degraded biologically.

Bemegride (β-ethyl-β-methylglutarimide) was introduced by Shaw, Simon, Cass, Shulman, Anstee and Nelson in 1954 as an antagonist of the effects of barbiturates. Conflicting statements have been made as to its specificity, effectiveness and toxicity when used for this purpose. Injected intravenously in animals or man in sufficient dosage (particularly as the soluble sodium derivative), it will induce convulsions, and in man non-convulsive doses may give rise to a period of restlessness and mental disturbance; if the animal or patient is in a comatose state induced by a barbiturate it may often bring about a rapid improvement in respiration, a rise in blood pressure and restoration of reflexes such as the righting reflex in rats and mice, or the withdrawal reflex on stimulating the sole of the foot in man. There is ample experimental evidence that "sleeping-time" and mortality from injected barbiturates in animals are much reduced by appropriate treatment with bemegride, but controversy persists as to whether or not the period of unconsciousness in man from a given barbiturate dosage is reduced. This doubt seems to be due largely to the failure by different authors to define the limits of consciousness and the type and blood level of barbiturate so that their work may be compared. The non-specificity of bemegride is no longer in doubt; it effectively rouses animals and patients narcotized by a variety of depressants of unrelated structure, but it has been effective in severe cases of barbiturate poisoning in man.

Relatively little seems to be known about the absorption and fate of this compound despite its use in therapeutics. Anderson (1958) described a spectrophotometric procedure capable of detecting $\beta\beta$-disubstituted glutarimides in biological fluids, and showed that bemegride disappeared rapidly from the blood stream after intravenous injection, 90% having gone within 20 min. This was attributed to a rapid and even distribution throughout the body and a slow rate of excretion in the urine. McCallum (1955) showed that bemegride was excreted by man partly unchanged and partly as β-(2-hydroxyethyl)-β-methyl-glutarimide.



A specimen (70 mg.) of bemegride labelled with ^{14}C at the two α positions was used to determine the distribution and fate of the drug. It had a specific activity of 8.5 μC./mg.

METHODS

The [^{14}C]bemegride was dissolved in saline to make a final concentration of 3 mg./ml. (25.5 μC./ml. activity).

Mice.—Groups of five male white mice of 20 to 25 g. wt. were injected intraperitoneally with pentobarbitone sodium 60 mg./kg. After 10 min. they were injected intravenously with 15 mg./kg.

[^{14}C]bemegride (approximately 2.5 μC./mouse). Each mouse was put at once into a glass chamber of 2 l. capacity through which 1.5 l. of air was pumped each minute. Food and water were provided after 4 hr. The outflow passed through a series of absorption tubes containing 10 N-NaOH. The trapped CO_2 was precipitated as barium carbonate, filtered, washed and suspended at a concentration of 100 mg./ml. for counting. The chamber was lined each time with fresh filter paper from which the urine was eluted with 50% aqueous methanol; any urine found in the bladder was added, and the whole dried, taken up in 2 ml. methanol/water and counted. The mice were removed after 5, 15 or 30 min. and 1, 2, 4, 8, 16 or 24 hr., decapitated and bled into a minimal volume of oxalate solution, 0.25 ml. from each being added to the pool from which samples were taken for counting. The carcasses were dissected immediately and the tissues rinsed in fresh saline to free them from blood, blotted dry, pooled by groups, weighed and stored at $-4°$ in plastic bottles. Brain, skin, fat, skeletal muscle, liver, lung, kidney, spleen, testis, thymus, small and large intestine wall, and washed out intestinal contents were collected. Soft tissues were ground with water to give a suspension containing 300 mg./ml. Muscle and scraped skin were hardened by immersion in liquid N_2, pulverized and ground up. Fat was extracted four times with 1 ml. 10% v/v methanol in chloroform with 30 min. shaking/extraction and the extract diluted with solvent to a concentration of 0.5 g. fat/ml. Free faeces were measured separately from the moist intestinal content, weighed, ground to a fine suspension of 300 mg. solid/ml. of water and counted.

Rats.—Four male hooded rats (210 to 240 g.) were fasted for 12 hr., dosed twice by stomach tube with 10 ml. warm water each at 1 hr. interval, injected intraperitoneally with pentobarbitone sodium 30 mg./kg. 30 min. later and the external jugular vein and the bladder cannulated. All urine was collected. 15 mg./kg. [^{14}C]bemegride (approximately 26 μC/rat) was injected intravenously 20 min. later and one rat killed at 15, 30, 60 or 120 min. Tissues and organs were dissected and prepared as for mice. Blood was separated into washed red cells, which were lysed in distilled water, and plasma. Protein was precipitated from the plasma by addition of 0.08 ml. of 50% trichloracetic acid/ml., centrifuged, washed three times and digested at 37° with 10 N-NaOH before counting.

Four female hooded rats in late pregnancy (230 to 270 g.) were similarly injected and killed after 15, 30, 60 or 120 min. Uterus, foetus, placenta and amniotic fluid were collected in addition to the tissues listed above.

Guinea-pigs.—Two males (240 to 360 g.) were anaesthetized with pentobarbitone sodium 60 mg./kg. intraperitoneally and the jugular vein and common bile duct were cannulated. After 20 min [^{14}C]-bemegride 7.5 mg./kg. (approximately 13 μC./animal) was injected intravenously and the bile collected at 15, 60, and 120 min.

Dogs.—Three male dogs were anaesthetized with pentobarbitone sodium 30 mg./kg. intravenously, the femoral vein cannulated and the cisterna magna punctured. A clear specimen of cerebrospinal fluid having been obtained as control, unlabelled bemegride sodium 20 mg./kg. was injected intravenously, and cerebrospinal fluid was again taken at 10 and 20 min. Bemegride was estimated by a modification of the colorimetric method of Sheppard, D'Asaro and Plummer (1956).

Rabbits.—Animals were anaesthetized with 25% urethane solution intravenously and a femoral artery cannulated. Bemegride sodium, 15 mg./kg., was injected intravenously, and cerebrospinal fluid, anterior chamber fluid and arterial blood were collected at 3, 5, 10 or 20 min. from three rabbits each. Bemegride was estimated by the method of Anderson (1958).

Radioactivity Estimations.—Scintillation counting using liquid phosphors was employed. The phosphor was 2:5-diphenyloxazole 7 g., *p*-di(5-phenyloxazol-2-yl)benzene 0.05 g., naphthalene 50 g., dissolved in 1:4-dioxan to 1 l., except for extract of fat, where the phosphor consisted of 4 g. *p*-terphenyl and 0.1 g. *p*-di(5-phenyloxazol-2-yl)benzene dissolved in 1 l. of toluene. The suspension (0.1 ml.) was pipetted into 5 ml. of phosphor solution in a glass dish, placed in the scintillator (Panax Universal USC/1), counting begun after 4 min. and approximately 10,000 counts taken. Phosphor decay due to visual light and count-rate decay due to settling of the suspension were minimal. All samples were counted in duplicate and recounted after adding an internal standard of 0.2 μC. [^{14}C]bemegride. The specific activity was expressed as μg. bemegride/g. tissue or as % total activity administered.

RESULTS

Distribution of Bemegride

Following intravenous injection bemegride is cleared rapidly from the blood, so that after 5 min. only some 12% of the initial dose remains. The rate of loss decreases and at 2 hr. 6% of the initial dose is still present. After 8 hr. the amount in the blood is very low (some 0.01% of the total initial dose) but persists for 24 hr. or more. Fig. 1 compares the blood levels found by us in mice with those given by Anderson (1958) for guinea-pigs. They are similar.

The bulk of the bemegride in the blood is in solution. Only some 0.02% of the total in plasma is precipitated with the protein. Since this small amount remains constant for 2 hr., during which time some 94% of the initial total has left the blood, it may be assumed to be protein-bound in a stable form. Very little bemegride penetrates the red cell envelope. Radioactivity was recorded from haemolysed red

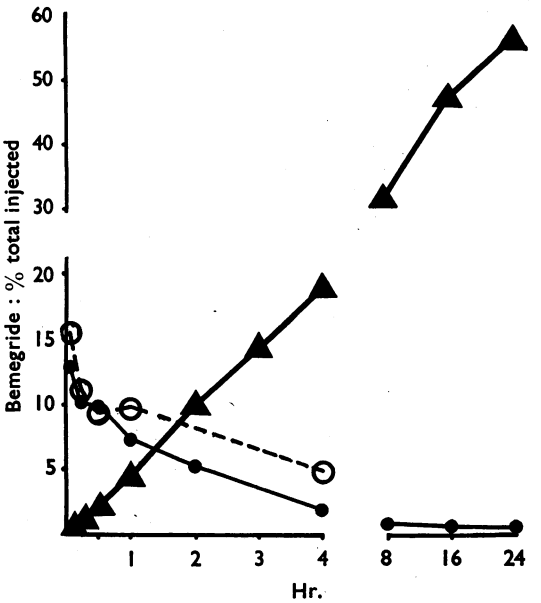


FIG. 1.—The concentration of [^{14}C]bemegride in blood (●) and urine (▲) of mice, expressed as % of the total intravenous injection (15 mg./kg.). Values for unlabelled bemegride in guinea-pig blood (O) are those given by Anderson (1958) for comparison. Note changes of scale in both axes.

TABLE I

DISTRIBUTION OF INJECTED [^{14}C]BEMEGRIDE IN RAT BLOOD

The concentrations at various times are expressed as μg. of bemegride/g. of tissue. Initial dose was 15 mg./kg. The amount in washed red cells or in the precipitated plasma protein (which may be bound and stable) remained constant over 2 hr. while the amount in whole plasma (probably free) fell steadily. The initial rapid fall was past before the estimates in this table were made.

Time (min.)	Plasma	Protein	Red Cells
15	21·7	0·87	0·29
30	19·2	0·89	0·56
60	18·7	0·96	0·30
120	13·0	0·89	0·28

cells, but as it only represented 0.007% of the total initial dose and as it remained more or less constant over the same period of 2 hr. the significance is doubtful; the activity might even be due to contamination of the cells by plasma or surface adsorption which resisted washing. These values for distribution within the blood after the initial rapid fall are shown in Table I, derived from the four male rats examined, expressed as μg./g. blood or blood fraction. In order to convert the values into percentages of initial total dose, the blood in the rat is taken as 7% of body weight.

The cause of the initial rapid fall in the concentration in the blood is shown in Table II, which gives the tissue concentration in mice at periods of time 5 min. to 24 hr. after intravenous injection. Bemegride passes rapidly into most tissues. Within a few minutes blood, brain, kidney, liver, spleen, and lung have similar concentrations, while those of the washed gut wall, fat, thymus, and testis are about 35 to 40% and skeletal muscle some 27% of that of the blood level. The skin value is low at 5%. This widespread distribution accounts for the rapid initial decrease in the concentration of bemegride in the blood. The ^{14}C activity is not found in the expired air and only increases in the urine at a steady rate. At 5 min. after injection into mice, when some 88% has left the blood only 0.06% is found in the urine. This increases steadily to 56% after 24 hr.

As with mice, this early and wide distribution is also found in rats of both sexes (see Table III); in addition it is evident that bemegride readily crosses the placental barrier and is found in the foetus in concentrations slightly above those in the maternal blood. It penetrates into amniotic fluid more slowly. The steadily increasing amounts in the intestinal content (upper small gut and whole gut washings in rats;

TABLE II

MEAN TISSUE CONCENTRATIONS IN GROUPS OF 5 MICE AFTER INTRAVENOUS INJECTION OF [^{14}C]BEMEGRIDE

15 mg./kg. of bemegride was injected and the concentrations of the drug at various times are expressed in μg./g. tissue or ml. blood. The urine and washings from gut lumen are expressed as % total dose. No $^{14}CO_2$ was detected in expired air at any time.

Time	Blood	Brain	Urine %	Kidney	Liver	Spleen	Lung	Gut	Gut Contents %	Muscle	Fat	Testis	Thymus
5 min.	27·3	23·9	0·06	25·3	33·7	23·5	17·4	9·8	0·5	7·2	11·7	11·3	10·6
15 ,,	22·7	14·9	0·8	30·8	36·1	52·9	38·7	13·8	1·1	7·4	10·6	11·5	10·3
30 ,,	19·5	12·5	1·8	29·6	26·8	44·7	25·2	13·1	1·9	6·9	10·5	9·7	8·7
1 hr.	15·8	10·0	4·4	28·5	30·3	51·4	36·0	11·9	1·4	9·8	7·1	10·0	13·8
2 ,,	11·8	8·3	10·2	22·9	22·4	31·8	23·6	11·4	2·2	7·9	7·7	11·6	17·3
4 ,,	4·2	4·7	18·8	22·6	15·4	34·7	15·1	4·4	0·7	2·7	2·5	5·3	8·6
8 ,,	1·5	4·6	30·7	6·7	6·9	2·6	13·7	2·7	0·4	1·1	0·6	2·3	4·6
16 ,,	0·2	2·7	46·8	4·8	4·6	2·9	8·2	0·7	0·4	0·3	0·5	0·7	2·8
24 ,,	0·2	2·8	56·1	5·9	6·1	8·0	4·1	1·0	0·4	0·4	0·5	0·8	2·9

TABLE III

TISSUE AND FLUID CONCENTRATIONS OF [^{14}C]BEMEGRIDE IN RATS WHEN INJECTED INTRAVENOUSLY

Concentrations are expressed in μg. bemegride/g. tissue or /ml. fluid. The urinary excretion at various times after the injection of 15 mg./kg. of bemegride is expressed as % initial dose. Each measurement was obtained from a single animal.

Time (min.)	Female Rats									Male Rats		
	Blood	Uterus	Placenta	Foetus	Amniotic Fluid	Liver	Kidney	Heart	Fat	Urine	Blood	Fat
15	20·5	6·4	16·5	24·6	7·5	26·9	19·6	14·6	5·2	0·3%	22·0	11·2
30	18·4	7·6	25·9	24·0	8·4	25·6	44·9	21·8	5·7	2·2%	19·8	12·9
60	17·1	10·0	21·2	24·1	9·2	27·8	29·7	24·3	5·0	2·7%	19·0	9·6
120	15·2	5·3	14·1	19·2	4·4	30·2	24·0	32·4	6·3	4·1%	13·3	6·7

total gut content of mice) are accounted for by the prompt excretion of bemegride in bile (see Table IV). In guinea-pigs, the quantity excreted by this route in 2 hr. amounts to 3.7% of that initially given. The biliary excretion (as measured from washings of the upper small intestine) would appear to be over more quickly in the rat than in the guinea-pig. The persistent difference between the concentrations from upper small intestine washings in male rats and the total gut content of female rats implies a sex difference in biliary excretion, or, more probably, excretion into the lumen in the initial phase when the blood concentrations are high, perhaps in digestive juices. The markedly lower concentration in fat than in blood found in mice is also found in rats.

Brain concentrations are similar to but somewhat lower than those in blood, implying that bemegride passes readily across the blood-brain barrier. This is supported by the evidence from rabbits shown in Table V. The arterial plasma concentrations in rabbits are similar to those in cisternal cerebrospinal fluid and in the aqueous humour in the anterior chamber of the eye (absorptiometric determinations). Calculation of the initial plasma concentrations in rats, mice, guinea-pigs, and rabbits from the expected blood volume and the known dose of bemegride indicate that the initial fall in concentrations of bemegride in blood is similar in all these species. The cerebrospinal fluid loses its bemegride somewhat more readily than does blood or anterior chamber fluid in the first half-hour, but brain as a whole retains it longer. The rapid early loss from cerebrospinal fluid may imply a passage into cells of the central nervous system.

The steady fall in the concentration of bemegride in blood and tissues and the rise in the urine continue after the initial rapid distribution. There is a tendency for the concentration in brain to decline more slowly than in other tissues. For example, at 8 hr. the blood concentrations decline to approximately 10% of those at 1 hr., and in liver, kidney, gut, and testis contents to approximately 20%, whereas the concentration in the brain only declines to 50%. Lung (approximately 40%) shows a similar tendency to retain bemegride, in contrast to the spleen (5%), another organ full of blood. There may be some reality, therefore, in the difference measured. After 24 hr. brain retains 28% of the concentration at 1 hr., kidney 21%, liver 20%, thymus 21%, spleen 15%, lung 11%, gut 8%,

TABLE IV

COMPARISONS OF RADIOACTIVITY IN BRAIN, BLOOD AND INTESTINAL CONTENTS OF MICE, RATS AND GUINEA-PIGS

The results are expressed as % of total intravenous dose of [^{14}C]bemegride, related to the weight of tissue or volume of fluid. The numerals in parentheses refer to the numbers of each species used.

Time (min.)	Mouse (5)	Male Rat (2)	Female Rat (2)	Guinea-pig (2)
	Whole Gut	*Proximal Gut*	*Whole Gut*	*Bile*
15	1·1	0·6	2·2	0·41
30	1·9	1·1	2·2	
60	1·4	1·0	2·7	2·1
120	2·2	0·3	2·4	3·7
	Brain	*Brain*	*Brain*	
15	2·1	1·2	1·6	
30		1·1	1·1	
60	1·1	0·9	0·9	
120	1·0	0·6	0·6	
	Blood		*Blood*	
15	10·9		9·6	
30	9·5		8·6	
60	7·6		8·0	
120	5·6		7·1	

TABLE V

CONCENTRATIONS OF UNLABELLED BEMEGRIDE IN ARTERIAL BLOOD PLASMA, CEREBROSPINAL FLUID AND AQUEOUS HUMOUR OF RABBITS AND IN CEREBROSPINAL FLUID OF DOGS

Both species were anaesthetized with urethane. Estimations, given in μg./ml., were made on rabbits using an absorptiometric method and in dogs with a colorimetric technique.

Time (min.)	Rabbit			Dog
	Plasma	C.S.F.	Aqueous Humour	C S.F.
3	48·7	43·2	40·1	—
5	41·2	31·8	30·4	—
10	36·0	23·0	31·1	37
20	29·1	21·0	32·5	12

testis 8%, fat 7%, muscle 4%, and blood 1.3%. One may conclude that all the tissues retain bemegride for a long period of time (24 hr.) and in a variable concentration. The central nervous system does so preferentially to a moderate degree whereas fat does not. This trend becomes clear if we examine the brain/blood ratios at various times (Table VI).

TABLE VI

THE BRAIN/BLOOD CONCENTRATION RATIOS OF [^{14}C]BEMEGRIDE AT VARIOUS TIMES AFTER INTRAVENOUS INJECTION IN MICE

A low concentration of bemegride is retained in the central nervous system for a long period.

Time:	(Min.)			(Hr.)					
	5	15	30	1	2	4	8	16	24
Blood/brain ratios ..	0·92	0·70	0·60	0·69	0·70	1·3	3·3	15	15

Excretion

An important pathway of excretion is in the bile; excretion in the other digestive juices is probable. It is difficult to say how much is reabsorbed from the intestine, but a small proportion, perhaps 5% of the total, is finally lost in the faeces in 24 hr. The main pathway of excretion is in the urine. No product of a totally degraded molecule escapes from the lungs. The rate of urinary excretion is slow, the peak rate occurring at 8 to 16 hr. after injection. Only some two-thirds of the initial dose has been excreted in 24 hr. by these channels. The rest would appear to be retained in the tissues. Direct evidence of accumulation has not been produced in this study but may occur with a suitable dosage; a daily injection of 10 mg./kg. to growing rats for 30 days produces no detectable toxic effect.

DISCUSSION

The absence of radioactivity in expired air makes it clear that the ring structure of bemegride is not drastically fragmented by metabolic degradation. This deduction is supported by the findings of Anderson (1958) whose method of estimation of bemegride is dependent upon an intact glutarimide structure, and the finding by McCallum (1955) of a hydroxylation product in urine. The rapid and widespread distribution may well account for the speedy onset of the effects of bemegride which are in contrast to the delayed action of picrotoxin. The pK_a of 11.2 (Wiggins, personal communication) indicates that bemegride exists in the blood largely in an unionized form. It has a low coefficient of distribution between arachis oil and phosphate buffer (*p*H 7.4 at 37°) of 3.2, and is very soluble in fat solvents such as ether and chloroform. The content of bemegride in fat found after injection into the blood is consistently low and examination of residues has confirmed that this is a true state of affairs and not a misconception due to poor extraction from fat. Bemegride must, therefore, penetrate cells by some other route than the lipophilic one if such exists. The work of Kahn (1952) with picrotoxin and that of Achor, Geiling, and Domek (1956) with bemegride on the distribution of [^{35}S] thiopentone sodium implies that bemegride decreases the concentration of barbiturate in the central nervous system, reduces its storage in fat, and increases the rate of excretion in urine, while picrotoxin has no effect on distribution. These changes may have been due to the saline injected. No controls are mentioned. If this effect (essentially one on membrane permeability) is indeed the basis of the mode of action it must be effective at low concentrations of bemegride, because antagonism to barbiturate can be demonstrated in mice or men for 2 hr. or more after injection, and at that time the concentration in the brain and cerebrospinal fluid is very low. Also this effect must modify the distribution of other sedative-hypnotics in a similar way. It seems unlikely. The retention of one-third of the initial dose after 24 hr. suggests the possibility of a long persisting clinical activity and a low toxicity.

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