Biochemical Pharmacology, 1965, Vol. 14, pp. 1225–1535. Pergamon Press Ltd., Printed in Great Britain.

STUDIES ON 6-AZAURIDINE AND 6-AZACYTIDINE—II.

THE EFFECTS OF 6-AZAURIDINE ON THE CENTRAL NERVOUS SYSTEM

I. JANKŮ, M. KRŠIAK, L. VOLICER, R. ČAPEK, R. SMETANA and J. NOVOTNÝ

Institute of Pharmacology, Czechoslovak Academy of Sciences,
Prague, Czechoslovakia

(*Received* 12 *April* 1965; *accepted* 21 *May* 1965)

Abstract—The effects of 6-azauracil and its ribonucleoside—6-azauridine—on the central nervous system were compared in mice. It was shown that loss of the righting reflex, motor inco-ordination and analgesia are rather subtoxic effects, the values for the ED_{50} being close to those of the LD_{50} of both compounds. The antagonism towards various convulsant agents appears to be more specific, especially as antagonism to nicotine is concerned. The most sensitive indicator of central activity of both 6-azapyrimidines was found in the depression of exploratory activity in mice and rats. Conditioned avoidance behaviour was unaffected at very high doses. In all these tests the difference between 6-azauracil and 6-azauridine was only quantitative, the ribonucleoside being 2–4 times less active on a molar basis than the free base; however, the 50 to 100-fold increase in the activity of 6-azauridine after its direct injection into the cerebral ventricles of mice and cats, in which a typical behavioural pattern is evoked, suggests that penetration through the hemato-encephalic barrier is an important limiting factor with respect to the potentiality of 6-azauridine for exerting an action on the central nervous system. Qualitatively identical effects have been observed after intraventricular injection of 6-azauridine-5′-monophosphate and small amounts of this nucleotide have been detected in various regions of the cat brain after administration of ^{14}C-labelled 6-azauridine into the cerebral ventricles. The possibility is discussed that central activity and carcinostatic effects of 6-azauracil and 6-azauridine are based on a common biochemical mechanism, namely inhibition of orotidylic acid decarboxylase resulting from the formation of 6-azauridine-5′-monophosphate.

6-AZAURACIL, a cytostatic antimetabolite of uracil, has strong depressant effects on the central nervous system that brought about its withdrawal from clinical therapy.[1] Some features of its pharmacology have recently been described by Welch, Handschumacher and Jaffe.[2] Among the toxic signs, "profound hypnosis, muscular relaxation and loss of reaction on painful stimuli," were noted in mice. On the other hand, 6-azauridine, the ribonucleoside of 6-azauracil, which according to Handschumacher *et al.*[3] is up to 20 times more active as a carcinostatic agent than 6-azauracil (on a molar basis), was found to be devoid of central activity, at least at therapeutic dosage. The lack of correlation between the cytostatic and neurotoxic properties of both 6-azapyrimidines has thus thrown doubt on the possibility that neurotoxic effects of 6-azauracil are based on the same biochemical mechanism as its cytostatic activity,[2] namely inhibition of orotidylic decarboxylase as a result of the formation of 6-azauridine-5′-monophosphate.[4]

It is known, however, that the penetration of 6-azauridine into the central nervous system is reduced very considerably as compared to 6-azauracil.[5, 6] Therefore, we felt it necessary to examine the effects of 6-azauracil in more detail and to find out whether similar effects could not be observed also with 6-azauridine in higher dosage. Simultaneously, it seemed worthwhile to study the effects of 6-azauridine after by-passing the hemato-encephalic barrier by introducing it directly into cerebral ventricles.

METHODS

The following criteria of central nervous activity were chosen for comparison of activity: loss of righting reflex, impairment of motor co-ordination, analgesic and anticonvulsant activity, the influence on exploratory activity and conditioned avoidance behaviour.

The loss of the righting reflex was tested simply by placing an animal on its back and the reaction was taken as positive if a reversal to the normal upright position did not occur within 30 sec.

Motor inco-ordination was assessed by the inability of the animal to stay 1 min on a rotating rod (2 cm diam.), revolving 4 times per min.

The hot-plate method was used for testing analgesic activity. The temperature of the plate was kept at 60° and the percentage of mice not licking their feet within 30 sec after the exposure was estimated.

The antagonism against various convulsant agents was studied on changes of the minimal amount of the convulsant necessary to produce death in the experimental animal. Convulsant agents were administered by means of a continuous intravenous infusion at a constant rate, 0·3 ml/min, using the technique described by Hint and Richter.[7] The following concentrations of convulsants were used: pentamethylenetetrazole, 1 per cent; strychnine nitrate, 0·005 per cent; nicotine bitartrate, 0·05 per cent; and arecoline bromide, 2 per cent. The infusion of convulsants was started at the time of the maximal effect of the compounds as determined on the rotating rod (90 min for 6-azauracil; 120 min for intraperitoneal and 15 min for intraventricular administration of 6-azauridine).

The effect on exploratory activity was tested according to the procedure of Lát[8] in rats and the same method was adapted to mice. In both cases the tests were performed in a box (24 × 24 × 15 cm for mice, 40 × 40 × 60 cm for rats) divided by a trapdoor into two unequal compartments. In the beginning of the session the animal was placed into the smaller compartment and after 5 min the trapdoor was opened so that the animal could enter the larger compartment. The behaviour of the animals was observed for 10 min in mice and for 13 min in rats. During this period the total times spent in exploring (walking, sniffing, rearing on hindlegs), as relevant activity, and in grooming (wiping of the face, licking, scratching), as irrelevant activity,[9] were recorded. The animals were divided into groups according to their individual exploratory activity, as determined in the first four sessions; animals with large variations of exploratory activity were discarded from the experiments. A groups of control animals was run parallel to the drug-injected group (test-group) in all experiments. In the test groups, sessions during which the compounds tested were injected were alternated with involving the sessions administration of saline; in the control groups only saline was injected. The evaluation occurred by comparing the percentage

change activity as a result of drug administration with the corresponding changes in the control group, using the nonparametric U-test of Mann and Whitney.

The influence of the compounds on learned behaviour was tested on avoidance conditioning in mice. Mice were trained to avoid an electric shock in a 30-cm long, straight runway. The opening of the starting box (11 × 12 cm) served as the conditioned stimulus and was followed 5 sec later by an electric shock (80 V) delivered to the feet of the animal through a stainless steel grid if the animal failed to reach the goalbox within this interval. Fifty trials at intervals of 30 sec were performed during one experimental session and 4 sessions were necessary for the 90–95 per cent acquisition of the conditioned response.

For the injection of 6-azauridine and 6-azauridine-5′-monophosphate into the lateral cerebral ventricles of mice a semiautomatic apparatus, especially constructed in our department by Krebs,[10] was used. Injection into the lateral cerebral ventricles in cats were performed using the cannula of Feldberg and Sherwood.[11] In some of these experiments the formation of 6-azauridine-5′-monophosphate in the cat brain was also examined. Two millilitres of a mixture of 50 mg 6-azauridine + 6-azauridine-4,5-^{14}C were administered into the left lateral ventricle of a cat. After 2 hr the animals were sacrificed, their brains quickly removed, washed with cold saline, weighed and frozen. The isolation and determination of radioactivity of 6-azauridine-4,5-^{14}C and its phosphate ester in the perchloric acid extract were performed by means of a slightly modified procedure previously described.[12, 13]

For intraperitoneal administration both compounds were dissolved in distilled water, the solution of 6-azauracil being stabilized by the addition of 5 per cent Tween 80. A constant volume, 1·0 ml, was always injected. In experiments in which 6-azauridine was administered into the cerebral ventricles, the compound was dissolved in freshly prepared Tyrode's solution and injected in a constant volume: 0·02 ml in mice and 0·5 ml in cats.

The pH of all solutions was carefully controlled and control experiments were performed with blank solutions acidified to the pH of the azauridine solution. In the majority of the experiments the evaluation of the effects occurred at the time of the maximal effect of the compounds on the rotating rod, i.e. 90 min after administration of 6-azauracil, and 120 min after intraperitoneal or 15 min after intraventricular injection of 6-azauridine. Acute toxicity and all quantal tests were evaluated using the graphic probit procedure of Litchfield and Wilcoxon.[14]

The experiments were performed primarily in 920 white mice (strain H, stock Konárovice), of both sexes, weighing 18–22 g; for each test and dose a group of at least ten animals was used. Exploratory activity was tested also on 36 white Wistar rat males, weighing 180–280 g. Fifteen cats were used for the intraventricular administrations of 6-azauridine.

RESULTS

The acute toxicity of 6-azauracil and 6-azauridine in mice, as well as their effect on preservation of the righting reflex, motor co-ordination on the rotating rod and on the reactivity on painful stimuli may be seen from Table 1. It appears that in all parameters studied, parallel to the action of 6-azauracil, an effect of 6-azauridine was found; however, the effectiveness of the ribonucleoside, on a molar basis, was less than that of the free base by a factor of 2·3 to 3·8. If compared to the LD_{50}-values, the ED_{50}

TABLE 1. COMPARISON OF SOME EFFECTS OF 6-AZAURACIL (AZU) AND 6-AZAURIDINE (AZUR) ADMINISTERED INTRAPERITONEALLY IN MICE

Observed effect	ED_{50}				Molar ratio AzUR/AzU
	AzU		AzUR		
	(g/kg)	(m moles/kg)	(g/kg)	(m moles/kg)	
Death	2·2 (1·65–2·93)*	19·5	11·25 (9·34–13·56)	45·9	2·35
Loss of righting reflex	1·55 (1·03–2·33)	13·7	10·5 (8·82–12·5)	42·8	3·12
Motor inco-ordination	0·90 (0·53–1·52)	8·0	5·2 (4·16–6·5)	21·2	2·65
Analgesia	0·70 (0·75–0·65)	6·2	5·8 (6·6–5·1)	23·6	3·80

* Limits of confidence for p = 0·95.

TABLE 2a. EFFECTS OF 6-AZAURACIL (AZU) ON INDIVIDUAL LETHAL DOSES OF VARIOUS CONVULSANTS IN MICE

AzU (mg/kg)	Pentamethylene-tetrazole (mg/kg)	Strychnine (mg/kg)	Nicotine (mg/kg)	Arecoline (mg/kg)
0	85·06 (98·8–73·16)*	0·94 (0·87–1·02)	5·69 (3·34–9·69)	187·8 (160·6–218)
100	112·46 (93·74–132·92)	1·03 (0·92–1·14)	5·43 (4·63–6·31)	212·0 (192·6–236)
200	113·96 (102·67–126·48)	1·25 (1·13–1·37)	11·31 (8·0–15·88)	206·0 (197–218)
400	104·9 (93·24–118·29)	1·38 (1·26–1·5)	13·00 (5·56–43·75)	256·0 (230–284)
800	166·61 (122·14–228·16)	1·69 (1·54–1·86)	20·63 (12·88–32·94)	326·0 (310–344)

* Limits of confidence for p = 0·95.

TABLE 2b. EFFECTS OF 6-AZAURIDINE (AZUR) INJECTED INTRAPERITONEALLY ON INDIVIDUAL LETHAL DOSES OF VARIOUS CONVULSANTS IN MICE

AzUR (g/kg)	Pentamethylene-tetrazole (mg/kg)	Strychnine (mg/kg)	Nicotine (mg/kg)	Arecoline (mg/kg)
0	99·1 (93·2–105·5)*	0·847 (0·812–0·876)	5·19 (4·95–5·44)	1·09 (0·99–1·2)
0·5	112·8 (103·7–122·6)	0·856 (0·817–0·896)	5·67 (4·96–6·42)	1·08 (1·02–1·13)
1·0	94·3 (88·3–100·8)	0·881 (0·854–0·910)	6·14 (5·72–6·59)	1·01 (0·97–1·05)
2·5	103·4 (95·0–112·5)	0·934 (0·896–0·972)	7·02 (6·53–7·53)	1·1 (1·05–1·15)
5·0	124·0 (116·5–131·9)	1·556 (1·437–1·685)	11·02 (9·96–12·19)	1·35 (1·24–1·47)

* Limits of confidence for p = 0·95.

data for motor inco-ordination and loss of reaction on painful stimuli represent, in both compounds, from 30 to 50 per cent of the LD_{50}. The righting reflex, as an indicator of "hypnotic potency", was impaired by even higher doses, which especially with 6-azauridine were very close to the mean lethal dose.

Table 2 summarizes our results with the effect of 6-azauracil and 6-azauridine on the lethal effects produced by various convulsant agents. The results indicate that both 6-azauracil and 6-azauridine may antagonize the effects of pentamethylenetetrazole, strychnine, nicotine and arecoline, although there are marked differences in the degree of this antagonism as can be seen from Fig. 1 and Fig. 2. The antagonism to nicotine

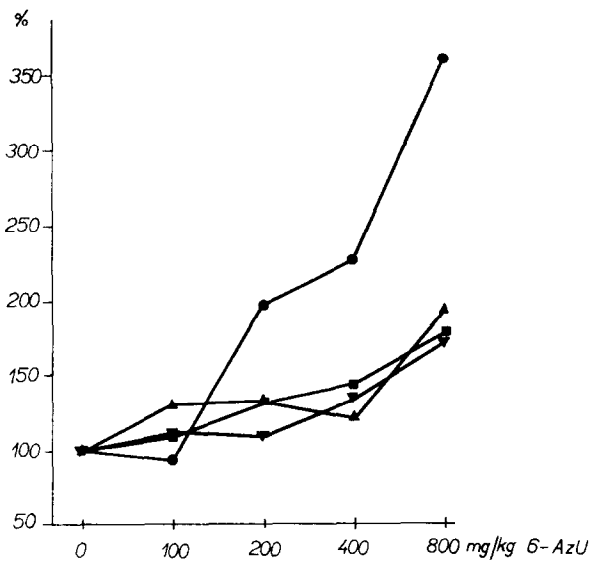

FIG. 1. Relative changes of mean individual lethal doses for nicotine ●——●, strychnine ■——■, pentamethylenetetrazole ▲——▲, and arecoline ▼——▼, after the intraperitoneal administration of 6-azauracil, expressed as percentage of the control values.

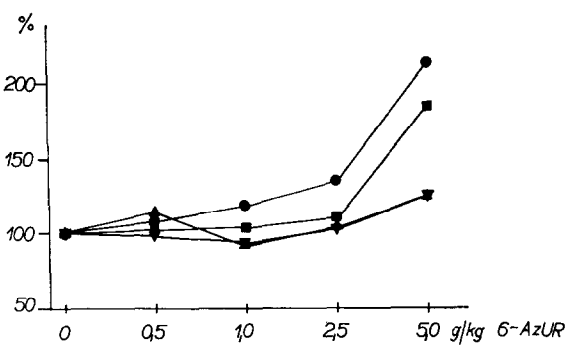

FIG. 2. Relative changes of mean individual lethal doses of nicotine ●——●, strychnine ■——■, pentamethylenetetrazole ▲——▲, and arecoline ▼——▼, after the intraperitoneal administration of 6-azauridine expressed as percentage of the control values.

appear to be the most striking in both instances, while strychnine, pentamethylenetetrazole and arecoline were much less effectively antagonized. Especially with 6-azauridine, with which the rise in the effective doses in general was smaller, statistically significant elevations of the effective doses of strychnine, pentamethylenetetrazole and arecoline were found practically only with the highest dose.

The exploratory activity of mice appeared to be affected notably by doses of 6-azauracil beginning with 12·5 mg/kg administered intraperitoneally, although statistically significant reductions in time spent exploring occurred at 50 and 100 mg/kg, with which exploration was depressed to approximately 50 per cent of the pre-test level. On the other hand, no statistically significant changes parallel to the effects on exploration occurred in the time spent grooming. 6-Azauridine was active at 100–200 mg/kg after either intravenous or intraperitoneal injection (Fig. 3). The quantitative comparison of the effects of 6-azauracil and its ribonucleoside on exploratory activity shows that, on a molar basis 6-azauracil has 2·3–2·8 times more activity than the ribonucleoside.

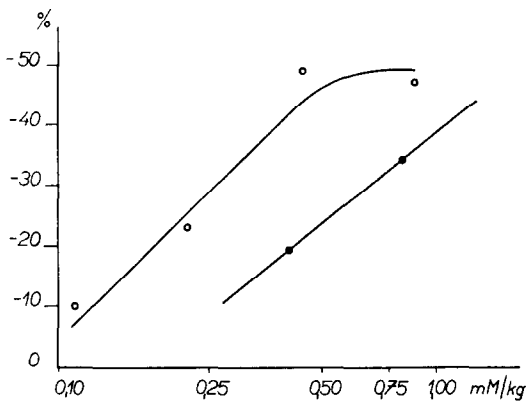

FIG. 3. Effects of 6-azauracil ○——○, and 6-azauridine ●——●, on exploratory activity in mice. The effects are expressed as the differences in the percentual changes of exploratory activity between the treated groups and control animals.

Similar results also were obtained on exploratory activity in rats. With repeated administration, however, the effect of 200 mg of 6-azauridine per kg gradually diminished until it ceased completely (Fig. 4). The rate of development of this tolerance varied from individual to individual and was fastest in rats of medium activity, whereas in either hyperactive or hypoactive animals the tolerance to the effects of 6-azauridine developed more slowly (Fig. 5).

In contrast to the results with exploratory activity, there was no deconditioning in mice trained to avoid an electric shock in a straight runway, even in very high doses of either 6-azauracil (500 mg/kg given intraperitoneally) or 6-azauridine 1500 mg/kg given intravenously), although with 3 mg of chlorpromazine per kg given intravenously the deconditioning was complete.

After administration into the lateral cerebral ventricles the activity of 6-azauridine was increased more than 50 times and typical changes in the motor behaviour of mice were observed with this route of administration. At very low doses (5–12·5 mg/kg intracerebrally) these changes were characterized first by rapid cleaning movements (wiping of the face, scratching), which after a short latency were almost regularly

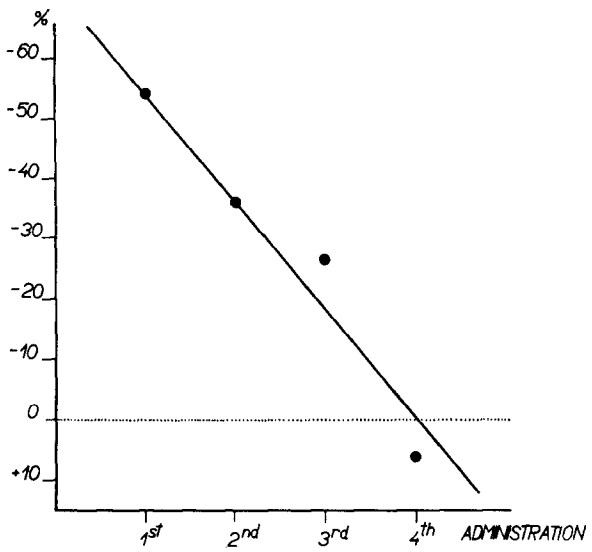

FIG. 4. Effect of repeated administration of 200 mg/kg of 6-azauridine intraperitoneally on exploratory activity of rats. The effects are expressed as the differences in the percentual changes of exploratory activity between the 6-azauridine treated group and control animals.

followed by bursts of jumping activity and by paroxysmal locomotion. Later, the locomotion became more or less inco-ordinated until it passed gradually into a series of muscular jerks or convulsions. With increasing dosage, however, this primary excitatory phase of the action of 6-azauridine was more or less replaced by a permanent general depression, during which some of the animals died. Identical changes in behaviour also were observed after the intraventricular injection of 6-azauridine-5'-monophosphate (1·0–5·0 mg/kg).

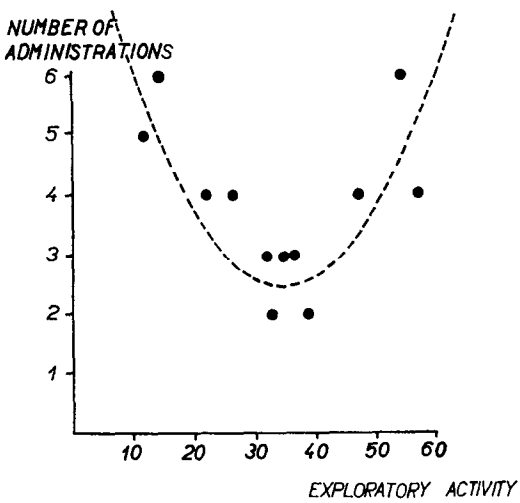

FIG. 5. Correlation between the individual exploratory activity of rats and the rate of tolerance development to depressant effects of 6-azauridine. The rate is expressed as the number of 6-azauridine administrations necessary to produce tolerance.

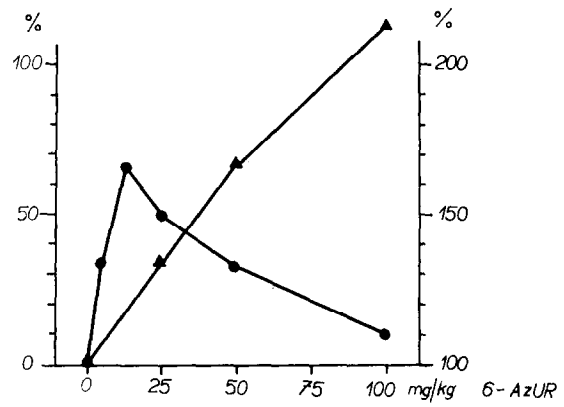

FIG. 6. Effects of 6-azauridine after injection into cerebral ventricles of mice on the frequency of paroxysmal locomotion ●——●, and on the relative change of mean individual lethal doses of nicotine ▲——▲.

The biphasic nature of 6-azauridine action, after its intraventricular injection, may well be seen in the frequency of the paroxysmal locomotion in relation to dose (Fig. 6). After reaching its maximum at 12·5 mg/kg intracerebrally, this phenomenon shows a gradual decline practically to zero when higher doses were injected. The figure also shows that with this decline there is a simultaneous increase in the antagonism to the effects of nicotine. The effects of 6-azauridine on motor co-ordination and mortality after injection into cerebral ventricles, with corresponding effects after intraperitoneal administration of the compound, are compared in Table 3.

TABLE 3. COMPARISON OF SOME EFFECTS OF 6-AZAURIDINE ADMINISTERED INTRAPERITONEALLY AND INTO CEREBRAL VENTRICLES IN MICE

Observed effect	6-azauridine g/kg intraperitoneally	6-azauridine g/kg intracerebrally	ratio i.c./i.p.
Death	11·25 (9·34–13·56)	0·22 (0·13–0·39)	0·0191
Motor inco-ordination	5·2 (4·16–6·5)	0·056 (0·01–0·14)	0·0108

Behavioural effects also were recorded after intraventricular injection in cats. In spite of a more limited dose range (25–50 mg/kg), the effects of 6-azauridine were essentially similar, although they emerged with greater plasticity. As in mice, there were various cleaning movements, including licking of the extremities and tail and scratching of the face, which sometimes was so violent that the animal remained blood-stained. In addition to that we observed various other stereotyped movements, such as repeated shaking of the head, pricking of ears, pawing or tail-lashing. The animals often tried to escape from the place of observation; in case of successful escape they walked or ran in a more or less co-ordinated way, although there was considerable ataxia in some subjects. Some animals also behaved as if they were unaware of their

environment, since they were not able to avoid obstacles in their way. On some occasions signs of aggressiveness also were present; thus some cats attacked surrounding inanimate objects or furiously bit parts of their own body. The locomotion was often interrupted or terminated by a sudden fall on the animal's side. Within 30 min after the injection practically all animals fell asleep.

The results of the experiments with ^{14}C-labelled 6-azauridine are summarized in Table 4. It can be seen that after the intraventricular administration 6-azauridine accumulates in varying amounts in different regions of the cat brain. Quantitatively,

TABLE 4. DISTRIBUTION OF ^{14}C-LABELLED 6-AZAURIDINE (AZUR) AND 6-AZAURIDINE-5'-MONOPHOSPHATE (AZUMP) IN DIFFERENT REGIONS OF CAT BRAIN AFTER THE ADMINISTRATION OF 50 MG (=203·9 μMOLES) OF 6-AZAURIDINE + 6-AZAURIDINE-4,5-^{14}C INTO THE LEFT LATERAL CEREBRAL VENTRICLE

Brain region	AzUR μmoles/g tissue	AzUMP μmoles/g tissue	$\frac{\text{AzUMP}}{\text{AzUR}} \times 100$
Cortex	0·218 (0·153–0·283)*	0·0096 (0·0088–0·0104)	4·40
Diencephalon	0·744 (0·679–0·809)	0·0170 (0·0162–0·0172)	2·28
Mesencephalon	0·588 (0·523–0·653)	0·0217 (0·0209–0·0255)	3·69
Cerebellum	0·449 (0·384–0·514)	0·0160 (0·0152–0·0168)	3·55
Medulla oblongata	0·539 (0·474–0·604)	0·0180 (0·0172–0·0188)	3·34

* Values in parentheses are limits of confidence for p = 0·95.

the following decreasing order of concentrations could be established: diencephalon > mesencephalon > medulla oblongata > cerebellum > cerebral cortex. Small quantities of ^{14}C-6-azauridine-5'-monophosphate also were detected; however, these did not exceed 2–5 per cent of the amount of 6-azauridine.

DISCUSSION

Our experiments with 6-azauracil confirm the previous results of Welch *et al.*[2] as regards the central depressant effects of this compound recorded in mice by rough qualitative observation. Quantitative comparison show, however, that the doses necessary for the production of the effects described by these authors are not very far from LD_{50}-values and thus cannot serve for a pharmacological classification of the compound. From this point of view the antagonism against various convulsants observed by us appears to be a more specific effect, especially as nicotine antagonism is concerned. These data speak for an anticholinergic component of 6-azauracil action, which with regard to its weak antiarecoline antagonism is directed more towards cholinergic N-receptors than M-receptors, in the sense of Aničkov.[15] The more intensive antagonism towards nicotine, as compared to pentamethylenetetrazole, is in contrast to the similarity of the chemical structure of 6-azauracil to that of barbiturates, as stressed by Wells *et al.*,[1] while on the other hand the reverse is true

for barbiturates.[16] The most sensitive indicator of the central activity of 6-azauracil, therefore, might be seen in the impairment of exploratory activity of mice and rats, in which species a dose of 50 mg/kg given intraperitoneally (corresponding approximately to 2 per cent of the LD_{50}) markedly inhibits this activity. Moreover, it seems that the depressive effects of 6-azauracil are in this case limited only to behaviour closely connected with exploration (walking, rearing on hindlegs), as at the same time no significant changes were observed in another activity of the animal (grooming). This selectivity of action also might be the reason for the apparent inactivity of the compound in influencing avoidance conditioned behaviour.

It is interesting to note that the same pattern of activity may be found also in 6-azauridine, although on a molar basis 2·3–3·8-fold higher doses are required for the production of the same effect. This is in marked contrast with the 8–20 times higher activity of 6-azauridine as a carcinostatic agent.[17, 18] It seems, however, that this contradiction has probably some connection to the reduced penetration of 6-azauridine through the hemato-encephalic barrier.[5, 6] This view finds further support in our results of experiments with administration of 6-azauridine into the cerebral ventricles of mice and cats, in which area this compound has been found effective in doses $\frac{1}{5}$th to $\frac{1}{100}$th those required after intraperitoneal injection. Neurological disturbances, as well as alterations in the EEG picture, also were observed after repeated administration of relatively small amounts of 6-azauridine into the ventricular system of the cat brain.[19]

As to the nature of these effects, it should be pointed out that a biphasic behavioural pattern is evoked, which in its excitatory phase resembles that accompanying audiogenic seizures in rats.[20] Scratching and licking also were observed by Feldberg and Sherwood[21] after injection of di-isopropylfluorophosphate (DFP) into the lateral cerebral ventricles of cats. However, it might be that this component of 6-azauridine action, not encountered after systemic administration, is due only to direct stimulation of brain structures situated close by the site of injection, where also the highest accumulation of 6-azauridine with this route of administration was found. The depressant component, on the other hand, is comparable to effects also observed after intraperitoneal injection.

It may be concluded, therefore, that neurotoxicity also is caused by 6-azauridine, but to a much lesser extent than with 6-azauracil, because of the much lower penetration of the hematoencephalic barrier by the ribonucleoside. This naturally cannot exclude the appearance of neurotoxic side-effects when permeability of the hemato-encephalic barrier is increased or when very large doses of 6-azauridine are administered. As our results on the influence of 6-azauridine on exploratory activity show, however, certain impairment of psychic activity also may occur with doses of this compound within the therapeutic range. On the other hand, the rapid development of tolerance towards these effects, which is in parallel to the developing resistance of leukemic cells growing in the presence of 6-azauridine,[22] probably would limit these side-effects to the initial period of 6-azauridine administration.

The quantitative comparison of the effects of 6-azauridine injected into cerebral ventricles with those obtained after intraperitoneal injection gave the following intracerebrally/intraperitoneally ratio—0·011, for motor inco-ordination, and 0·019 for lethality, i.e., values of the same order of magnitude as CSF/blood ratio, 0·013, found for 6-azauridine by Cardoso and Symmes.[6] If the reduced penetration of the

brain by 6-azauridine is taken into consideration, however, it is clear that the diminution of neurotoxicity of 6-azauridine, as compared to 6-azauracil, is less than would correspond to its lower access to the site of action in the brain. For with one-thirty-fourth as much penetration of 6-azauridine as with 6-azauracil,[6] we obtained only a 2·3 to 3·8-fold reduction of central activity. These figures show, therefore, that the "true" relative activity of 6-azauridine in comparison to 6-azauracil varies between 8–14, and suggest a higher potency of the ribonucleoside, parallel to its carcinostatic activity also in the brain.

The existing correlation between carcinostatic and central activities of 6-azauracil and its ribonucleoside is thus only masked by different levels of the two compounds in the brain. As 6-azauridylic acid, whose formation in the brain *in vivo* has been reported by us, as well as by Wells, Gaines and Koenig, also was active in our experiments, there is now evidence suggestive that carcinostatic and central activities of both 6-azapyrimidines might be related to the same biochemical mechanism—namely inhibition of orotidylic acid decarboxylase. This view is supported by the recent finding of Wells, Gaines and Koenig[19] showing that 6-azauridine really interferes with the formation of uridine nucleotides in the cat brain. The functional significance of such inhibition emerges from the observation of Geiger[23, 24] that uridine and cytidine are necessary for maintenance of normal activity of the brain. On the other hand, it is not yet clear whether hypnotic effects of certain 5-alkylderivatives of 6-azauracil described by Welch *et al.*[2] might be explained in the same way since these compounds have been found devoid of carcinostatic activity. Nevertheless, it should be considered that the high liposolubility of these derivatives, increasing with the length of the alkyl-chain, should even more affect their distribution in the body favouring predominantly the brain and adipose tissue.

Acknowledgement—We wish to thank Messrs. Spofa, United Pharmaceutical Works, Prague, for supplying us with 6-azauracil and 6-azauridine and to Academician F. Šorm, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, for the sample of 6-azauridine-5′-monophosphate.

REFERENCES

1. C. E. Wells, C. Ajmone-Marsan, E. Frei, J. H. Tuohy and B. I. Shnider, *Electroenceph. clin. Neurophysiol.* **9**, 325 (1957).
2. A. D. Welch, R. E. Handschumacher and J. J. Jaffe, *J. Pharmac. exp. Ther.* **129**, 262 (1960).
3. R. E. Handschumacher, S. Cardoso, J. J. Jaffe, A. A. Liebov, P. Calabresi, S. C. Finch and A. D. Welch, *Proc. Am. Ass. Cancer Res.* **3**, 116 (1960).
4. R. E. Handschumacher and C. A. Pasternak, *Biochim. biophys. Acta* **30**, 451 (1958).
5. V. Habermann and F. Šorm, *Coll. Czechoslov. Chem. Comm.* **23**, 2201 (1958).
6. S. S. Cardoso and D. Symmes, *Fedn Proc. Fedn Am. Socs exp. Biol.* **20**, 322 (1961).
7. H. C. Hint and A. W. Richter, *Acta Pharmacol. et Toxicol.* **14**, 153 (1958).
8. J. Lát, E. M. Widdowson and R. A. McCance, *Proc. R. Soc.*, **B153**, 347 (1960).
9. D. Bindra, *Psychol. Rep.* **11**, 307 (1962).
10. V. Krebs, *Proc. XXII. Internat. Physiol. Congr. Leyden*, Vol. II, DFT 25 (1962).
11. W. Feldberg and S. L. Sherwood, *J. Physiol.* **120**, 3 (1953).
12. R. E. Handschumacher, *J. biol. Chem.* **235**, 764 (1960).
13. R. E. Handschumacher, J. Škoda and F. Šorm, *Coll. Czechoslov. Chem. Comm.* **28**, 2983 (1963).
14. J. T. Litchfield, Jr. and F. Wilcoxon, *J. Pharmac. exp. Ther.* **96**, 99 (1949).
15. S. V. Anichkov, *Zh. vyssh. nerv. dejat.* I. P. Pavlova **12**, 391 (1962).
16. G. Chen and B. Bohner, *Archs int. Pharmacodyn. Thér.* **125**, 1 (1960).
17. J. J. Jaffe, R. E. Handschumacher and A. D. Welch, *Yale J. biol. Med.* **30**, 168 (1957).

18. F. ŠORM and H. KEILOVÁ, *Experientia* **14**, 215 (1958).
19. W. WELLS, D. GAINES and H. KOENIG, *J. Neurochem.* **10**, 709 (1964).
20. D. B. LINDSLEY, F. W. FINGER and CH. E. HENRY, *J. Neurophysiol.* **5**, 185 (1942).
21. W. FELDBERG and S. L. SHERWOOD, *J. Physiol.* **125**, 488 (1954).
22. C. A. PASTERNAK, G. A. FISHER, R. E. HANDSCHUMACHER, *Cancer Res.* **21**, 110 (1961).
23. A. GEIGER, J. MAGNES, R. M. TAYLOR, M. VERALLI, *Am. J. Physiol.* **177**, 138 (1954).
24. A. GEIGER and S. YAMASAKI, *J. Neurochem.* **1**, 93 (1957).