Anal. Calcd. for $C_9H_8Cl_2N_4O$: C, 41.7; H, 3.1; N, 21.6. Found: C, 41.8; H, 3.3; N, 21.4.

2-Amino-6-benzylthio-9-methylpurine.—To a stirred solution of 150 ml. of concentrated aqueous ammonia, containing 7.0 g. of 2-amino-9-methyl-6-purinethiol,[16] was added 20 ml. of *p*-dioxane and 5.0 g. of benzyl chloride. This mixture was stirred and heated (40°) for 1 hr. during which time the product precipitated from the solution and was filtered, washed with water, and dried. The crude product was recrystallized from ethyl acetate–petroleum ether (60–110°) to give 3.6 g. Recrystallization from benzene provided a sample with a melting point of 131–133°. Ultraviolet spectra: pH 1, λ_{max} 321 mμ, ϵ_{max} 11,700; pH 11, λ_{max} 313 mμ, ϵ_{max} 12,500; MeOH, λ_{max} 313 mμ, ϵ_{max} 12,500.

Anal. Calcd. for $C_{13}H_{13}N_5S$: C, 57.7; H, 4.8; N, 24.2. Found: C, 58.2; H, 5.1; N, 23.9.

2-Amino-6-benzylthio-7-methylpurine.—2-Amino-7-methyl-6-purinethiol[7] (150 mg.) was suspended in 5 ml. of water, and enough *N* sodium hydroxide was added to the suspension to effect solution. Benzyl chloride (3 drops) was added to this solution, and the solution was shaken at room temperature for 15 min. and cooled in an ice bath. The product was filtered and washed with a small volume of water (yield 100 mg.). Recrystallization was accomplished from benzene to give a product, m.p. 192–193°. Ultraviolet spectra: pH 1, λ_{max} 322, 280 mμ, ϵ_{max} 14,900, 10,300; pH 11, λ_{max} 323 mμ, ϵ_{max} 11,000; MeOH, λ_{max} 326 mμ, ϵ_{max} 11,000.

Anal. Calcd. for $C_{13}H_{13}N_5S$: C, 57.7; H, 4.8; N, 24.2. Found: C, 57.4; H, 4.7; N, 24.7.

Solvents Used in Chromatography.—The following three solvent systems were employed throughout this work: (1) 5% ammonium bicarbonate in water; (2) water saturated with *n*-butyl alcohol; (3) 5% disodium hydrogen phosphate in water saturated with isoamyl alcohol.

The Biological and Physical Properties of the Azaindoles

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Incorporation of an isoelectronic nitrogen atom in place of a methine group in the indole molecule increases the basic strength and reduces the lipid–water distribution ratio. Depending on the position of the doubly bonded nitrogen atom, the azaindoles exert pharmacological effects on smooth muscle and the central nervous system that either mimic or oppose the actions of indole.

Pharmacological interest in the azaindoles stems from the possibility that they may serve as the parent nucleus of active or antagonistic analogs of naturally occurring indole derivatives such as the endogenously important serotonin, melatonin, and tryptophan[2-4] and the potent pharmacological agents such as psilocybin and lysergic acid diethylamide. Furthermore, it seemed likely that unsubstituted azaindoles would have pharmacological properties because (unsubstituted) indole has marked pharmacologic effects: it depresses smooth muscle and produces convulsions originating in the spinal cord and subthalamic areas of the brain.[5-9] Of the six isomeric monoazaindoles, only one, benzimidazole (3-azaindole), has been studied *in vivo*: it was found to produce a transient flaccid paralysis owing to interneuronal depression.[10-13] Because virtually nothing is known of the biological actions of other mono- and polyazaindoles, their acute pharmacological effects in mice and their *in vitro* effects on smooth muscle have been determined in the present study. The determination of the lipid–water solubility ratio of the nonionized form, and the calculation of the degree of ionization at body pH, are also included in this paper because these physical properties are known to have a marked influence on the activity of centrally active agents.[14,15]

Experimental

Materials.—Commercial preparations of indole, 2-azaindole (1H-indazole), 3-azaindole (benzimidazole), 7-azaindole (I) (1H-pyrrolo[2,3-b]pyridine), and 2,3-diazaindole (benztriazole) were purified by recrystallization from hot water followed by high vacuum sublimation. All other mono- and polyazaindoles used in this work were synthesized in the Department of Medical Chemistry of the Australian National University, Canberra and their purification has been described.[16-19] The compounds include: 4-azaindole (1H-pyrrolo[3,2-b]pyridine); 5-azaindole (1H-pyrrolo[3,2-c]pyridine); 6-azaindole (1H-pyrrolo[2,3-c]pyridine); 3,4-diazaindole (1H-imidazo[4,5-b]pyridine); 3,5-diazaindole (1H-imidazo[4,5-c]pyridine); and purine (7H-imidazo[4,5-d]pyrimidine).

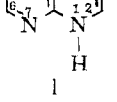

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TABLE I

PHYSICAL AND BIOLOGICAL PROPERTIES OF AZAINDOLES

Compound (A)	pK_a (basic) (B)	% ionized at pH 7.4 (C)	Lipid-water distribution ratio (D)	*In vivo* effects after i.p. injection in mice: Gross effects (E)	ED_{50}[a] mg./kg. (F)	LD_{50}[a] mg./kg. (G)	*In vitro* effects on smooth muscle: Rat stomach fundus Concn. ($10^{-4}M$)	Effect (H)	Guinea pig ileum Concn. ($10^{-4}M$)	Effect (I)
Indole	−2.4	0.0000001	85.7	Convulsions	185 (170-202)	316 (266-376)	0.4	Very marked relax.	0.4	Very slight relax.
2-Azaindole (indazole)	1.22	.00009	41.5	Mixed effects	70 (54-91)	440 (326-595)	.5	Mod. relax.	.5	Very slight relax.
3-Azaindole (benzimidazole)	5.53	1.33	16.4	Paralysis	195 (174-218)	640 (587-697)	.5	Mod. relax.	.5	Slight relax.
4-Azaindole	6.94	27.75	13.1	Convulsions	36 (32-41)	260 (221-306)	.8	Mod. relax.	.8	Slight relax.
5-Azaindole	8.26	87.87	17.3	Convulsions	6.3 (5.2-7.6)	16.5 (14.8-19.0)	3.9	Marked contraction	.9	Marked cont.
6-Azaindole	7.95	78.02	36.8	Convulsions	5.9 (4.6-7.5)	12.0 (10.0-14.4)	2.2	Marked contraction	1.1	Marked cont.
7-Azaindole	4.59	0.15	53.2	Paralysis	166 (148-187)	490 (445-539)	1.1	Slight to mod. relax	1.1	Mod. relax.
2,3-Diazaindole (benztriazole)	1.6	.0002	3.0	Mixed effects	250	500				
3,4-Diazaindole	4.0	.4	0.93	Convulsions		>200				
3,5-Diazaindole	6.1	4.76	1.03	Paralysis	>41	<200				
3,5,7-Triazaindole (purine)	2.4	.001	0.36	"Shock"		<150				

[a] Confidence limits (19/20) given in parentheses.

Lipid-Water Solubility Ratio.—Aqueous concentrations before and after equilibration with oil were determined by measuring fluorescence in an Aminco-Kiers spectrophotophosphorimeter adapted for fluorometry (American Instrument Company, Maryland). The oily phase used was oleyl alcohol previously purified by shaking with charcoal and distilling at 181° (1 mm.). Weighed amounts (1–10 mg.) of the compounds were dissolved in suitable buffers, that is at least 2 pH units above the respective pK_a to ensure that essentially only the neutral molecular form was present. After shaking for 10 min. at 25° (at least double the time required for equilibration) with an equal volume of oleyl alcohol, the phases were separated by centrifuging at 2000 r.p.m. and the oleyl alcohol removed by suction. The aqueous phase was diluted with buffer until a linear relationship between fluorescence and detector response was observed. The readings were corrected for the respective buffer blank contribution to fluorescence. The fluorescence characteristics of the azaindoles have been reported elsewhere.[20]

Ionization.—The extent of ionization at body pH was calculated from the pK_a values previously determined[16] and the relationship[21]

$$\% \text{ ionized} = \frac{100}{1 + \text{antilog}\,(7.40 - pK_a)}$$

Pharmacological Effects.—To study the *in vivo* effects, male Swiss-Webster mice weighing between 15 and 20 g. were used. All monoazaindoles were dissolved or finely suspended in cottonseed oil and administered intraperitoneally in volumes which did not exceed 0.25 ml. per 10 g. body weight. Dose-response curves were obtained using groups of 8–10 mice at each of 4 or 5 dose levels, and the median effective dose (ED_{50}) and median lethal dose (LD_{50}) together with their 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon.[22] Inasmuch as the acute effects of some compounds were depressant and others convulsant, the end points chosen for determining the ED_{50} were, respectively, the inability of the mouse to right itself when placed on its back and the onset of mild clonic-tonic convulsions. The survival times after toxic doses of the convulsant agents were compared with those of a group of mice receiving intraperitoneal injections of a solution of strychnine base dissolved in oil.

Owing to their extremely low oil solubility, 3,4-diazaindole, 3,5-diazaindole, and purine were dissolved in propylene glycol rather than cottonseed oil. Only qualitative observations were made since comparatively high doses were required and the amounts of compounds available were limited.

To study the effects on smooth muscle the rat stomach fundus strip preparation according to Vane[23] was used. The organ bath had a capacity of 50 ml. and the tissue was bathed in oxygenated Tyrode solution containing scopolamine hydrobromide ($10^{-7}M$). Similar experiments were performed on the guineapig ileum.

Results and Discussion

The biological and physical properties of the azaindoles are summarized in Table I. Among the monoazaindoles only the 4-isomer resembles indole in producing depression of smooth musle as well as stimulation of the central nervous system. The other monoazaindoles share either one or the other of these pharmacological properties; thus 3-aza- and 7-azaindole produce depression (and 5-aza- and 6-azaindole produce stimulation) of both systems; 2-azaindole causes depression of smooth muscle but produces mixed stimulant and depressant effects in the central nervous system.

Pharmacological Effects *in Vivo*.—The acute effects following intraperitoneal injection of the azaindoles are given in columns E-G of Table I. The toxic effects of all the mono- and diazaindoles appeared to be primarily of central origin, characterized either by the sudden onset of tonic- or clonic-tonic convulsions or by progressive flaccid paralysis followed by respiratory failure. Small doses of some of the compounds (indazole, benztriazole, and 3,5-diazaindole) resulted in mixed effects appearing as occasional clonic seizures (about 10/min.) superimposed on a flaccid paralysis sufficient to abolish the righting reflex. Increasing the dose in these cases produced death either by tetanic tonic convulsions (indazole) or respiratory failure (3,5-diazaindole), or by either of these with almost equal frequency (benztriazole). Although immediately after injection of most of these compounds a marked blanching of the ears was frequently observed, this was a transient effect and within 15 sec. the paws, snout, and tail of the mice were quite pink, suggesting that the circulation had returned to normal. With toxic doses of purine, on the other hand, the severe depression appeared to stem from a profound and prolonged circulatory collapse as

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evidenced by the cyanotic appearance and coldness to touch of the whole mouse. Death occurred 22 hr. later, at a time after respiration had returned to normal. It is possible that renal failure may be implicated in the delayed death with purine since the compound is metabolized to 2,8-dihydroxypurine[24] and it has been shown that renal tubular occlusion occurs from crystalline deposits of 2,8-dioxyadenine formed during *in vivo* metabolism of adenine,[25] isoguanine, or 2-oxyadenine.[26]

The central effects of the 2-, 4-, 5-, and 6-isomers of the monoazaindoles resemble those of indole in that all of them produce clonic-tonic convulsions at all doses (except for indazole which shows mixed stimulant and depressant effects at low doses). Particularly striking is the greatly increased potency of 4-, 5-, and 6-azaindole. With 4-azaindole, although the LD_{50} is not significantly different from indole, increased potency is seen at nonlethal doses where the ED_{50} is about 20% that of indole. 5-Azaindole and 6-azaindole are approximately 0.2 as potent as strychnine (which under the same conditions had an LD_{50} of 2.4 mg./kg.) and are between 20 and 25 times more toxic than indole; at nonlethal levels they are 30 times more effective than indole in producing convulsions. Also noteworthy is the extremely rapid onset of lethal action with 5-azaindole and 6-azaindole. No obvious hyperemia of the gut was apparent on autopsy, suggesting that local vasodilation was not responsible for the rapid absorption. The mean survival time after an interperitoneal injection of the LD_{95} dose of 5-azaindole was 8.2 min. (range: 5–17 min.) and 5.6 min. (2–15 min.) for 6-azaindole.

The remaining monoazaindoles, *viz.*, benzimidazole and 7-azaindole, are depressants causing progressive flaccid paralysis and eventual respiratory depression. It is interesting to note that these isomers share a common structural feature in having the singly bonded nitrogen atom separated by one carbon atom from the doubly bonded nitrogen. The paralytic action of 7-azaindole in mice is greatly prolonged compared with that of benzimidazole. The effects of benzimidazole at the calculated ED_{95} (225 mg./kg.) lasted between 20 and 70 min., whereas recovery from an equipotent dose of 7-azaindole (200 mg./kg.) took between 4 and 5 hr. While benzimidazole paralysis has been shown to be due to interneuronal blockade,[10,11] it must be noted that 7-azaindole may not have the same site of action because the interference with the righting reflex is a nonspecific effect that could result from curarenimetic or hypnotic properties. Attempts to differentiate the actions of 7-azaindole on monosynaptic (knee jerk) versus polysynaptic (flexor and crossed extensor) reflexes in the spinal rabbit were abandoned when it was found that intravenous injection of the compound reduced the blood pressure to shock levels. However, in the experiments with rats, no hypnotic action was apparent and many of the animals exerted weak but coordinated muscular movements when placed in a prone position even though unable to right themselves from a supine position. Furthermore, preliminary experiments in the frog indicated that no curaremimetic effect occurs because, after injection of 7-azaindole into the dorsal lymph sac, contraction of the gastrocnemius muscle could still be evoked by stimulation of the sciatic nerve. (It was noted that topical application of 7-azaindole to the frog sciatic nerve caused a reversible nerve-block, an effect which appears to be nonspecific inasmuch as it was also produced with two convulsant agents having high lipid–water solubility ratios, *viz.* indole and indazole.) The severe tonic-clonic convulsions produced by pentylenetetrazole (55 mg./kg.) were practically completely abolished in 4 out of 4 mice by pretreatment with 122 mg./kg. 7-azaindole. Because 7-azaindole may be regarded as 2-vinylaminopyridine, it has some chemical resemblance to the 2-ethylaminopyridine derivative that has been found to have central muscle-relaxing properties.[27]

Some additional observations on the pharmacological properties of 7-azaindole merit attention, particularly the delayed toxic effects. While death usually occurred between 1 and 5 hr. after injection of the LD_{95}, with smaller doses (near the LD_{50}) many of the mice that appeared to recover from the acute depressant effects gave evidence of more prolonged intoxication by dying 24 to 48 hr. later. The LD_{50} for acute deaths (less than 6 hr.) listed in Table I was equivalent to the LD_{95} for total deaths (acute plus delayed). No obvious reason for the delayed deaths was revealed by gross examination of the tissues on autopsy. The lungs, liver, heart, and intestines appeared normal although the kidneys, while normal in appearance, had a mean weight of 1.4% of the body weight. Too few measurements were made to determine whether this was significantly higher than the mean of 1.05% body weight for kidneys from untreated mice, and experiments are now in progress to determine whether the delayed deaths resulted from renal failure. Some preliminary experiments with 6-methyl-7-azaindole (m.p. 139–140°)[28] suggest that delayed toxicity does not occur when the 6-position is blocked; this may be analogous to situations in which renal failure is prevented by blocking the corresponding position in a purine, *viz.* 2-amino-adenine (4,6-diamino-3,5,7-triazaindole).[29] However, 6-methyl-7-azaindole was much less potent than 7-azaindole and had a very short duration of action. It is interesting to contrast the pharmacologically weakening influence of the 6-methyl group in 7-azaindole with the influence of the 6-methyl group in potentiating the paralyzing action of benzthiazole.[30]

Pharmacological Effects on Smooth Muscle.—Columns H and I of Table I list the *in vitro* actions of the azaindoles; it can be seen that indole and the 2-, 3-, 4-, and 7-azaisomers all produce relaxation of the rat stomach fundus strip and of the isolated guinea pig ileum in a concentration of approximately 5 to 10 μg./ml. While all these compounds antagonize the effect of 5-hydroxtryptamine (5-HT) on the guinea pig ileum, in certain instances (*viz.*, with 3-aza- and 4-azaindole) the fundus strip was rendered more sensitive to 5-HT. Stimulation of smooth muscle evoked by 25–50 μg. 5-aza- and 6-azaindole per ml. resembles that pro-

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duced by 5-HT in that the contraction is prevented by previous addition of 2-bromolysergic acid diethylamide (1.4 μg./ml.) to the bath. Others have found, however, that 5-azaindole does not closely resemble 5-HT inasmuch as the latter depresses orthodromic excitation of neurones of the lateral geniculate nucleus of the cat, while the former does not.[31]

Physical Properties of the Substances.—It will be shown that neither the pK_a values nor the lipid–water distribution coefficients should favor such rapid uptake by the brain cells of the 5- or 6-isomer compared with indazole and indole, which, act more slowly and are less potent. (After LD_{95} doses of indole or indazole the mean survival times were, respectively, 59.8 min. (44–73 min.) and 33.9 min. (27–49 min.).) Thus, the physical properties of the azaindoles (listed in Table I) are not well correlated with biological action, although they are well correlated with structure. In general, the incorporation of a doubly bonded nitrogen atom anywhere in the indole molecule confers basic properties on the molecule (where practically none exist in indole) and reduces the lipid–water distribution ratio. While all azaindoles are capable of forming cations under suitable conditions, the pK_a values of those isomers having both nitrogen atoms in the 5-membered ring (also those of all the polyazaindoles) are not high enough to cause appreciable ionization at pH 7.40. Hence these compounds are present almost completely as the free bases in body fluids. This is also true of 7-azaindole, while the other benzene-azaindoles show a fairly high degree of ionization, especially the 5- and 6-isomers, where, respectively, 88 and 78% exist as cations at body pH. Because 5- and 6-azaindole have an extremely rapid onset of action in the intact animal, it may be inferred that (despite the extensive ionization of these isomers) an effective number of neutral molecules gain ready access to the cells of the central nervous system.

The marked potency of the most basic isomers suggests that the cationic form of the convulsants is the effective agent, once the substance reaches the central neurones. On the other hand, a wide range of ionization ratios (from 28% to practically 0%) exists for 4-azaindole, 2-azaindole, and indole, yet their toxicities are not significantly different from each other. Moreover, the pK_a offers no clue as to which isomers will produce paralysis as opposed to convulsions. It appears, therefore, that the degree of ionization *per se* cannot be correlated directly with the biological action of the azaindoles.

The reduction in the lipid–water distribution ratio is one of the most striking and consistent effects of the aza-nitrogen atom. This reduction depends primarily on the number of extra nitrogen atoms in the indole structure and secondarily on their position relative to each other. Among the monoazaindoles when the nitrogen atoms are clustered together, as in indazole or 7-azaindole, the lipid–water partition ratio is about one half of that of indole; when the nitrogen atoms are separated by several carbon atoms the value drops to about one sixth. In the diazaindoles the values are less than one twenty-fifth that of indole, even when the nitrogens are adjacent to each other as in benztriazole. When the heteroatoms are spread throughout the molecule, as in 3,4- and 3,5-diazaindole, the lipid–water distribution ratio is less than one fiftieth that of indole. The combined effects of number and position are seen in the lipid–water distribution ratio of 3,5,7-triazaindole (purine), which is 250-fold lower than that of indole.

It is apparent from Table I that, while the lipid–water distribution ratios of the monoazaindoles are considerably less than that of indole, they are, nonetheless, all greater than 10 and hence should not be a limiting factor in the biological action. Thus, the isomer with one of the lowest partition ratios in this series, 5-azaindole, is one of the fastest acting and most potent convulsants. Furthermore, since a fairly rapid onset of action characterizes the effects of benztriazole or 3,5-diazaindole, it appears that rapid penetration into the brain is achieved when the lipid–water partition ratio is as low as one. One may conclude, therefore, that while there is almost a 100-fold range in the lipid–water distribution coefficients among the mono- and diazaindoles, the differences found are not correlated with the differences in biological activity because even the lowest of the lipid–water partition ratios is apparently high enough to facilitate rapid distribution to the brain.

With purine, on the other hand, the partition ratio is only 0.36 and this appears to be low enough to prevent rapid uptake by the brain with the result that the acute toxicity stems from peripheral rather than central action.

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