moderate success of potassium chloride in both *in vitro* and *in vivo* experiments on this special strain of mouse indicates that further investigation into the role of potassium as well as other ions should be carried out.

CONCLUSIONS

1. Methimazole-, digoxin-, and norethandrolone-treated rats of both sexes fed on a vitamin E-deficient diet demonstrated greater ability to perform forced motor activity than the untreated control groups.

2. Norethandrolone, digoxin, and methimazole increased the offspring survival rate of vitamin E-deficient female rats.

3. There appeared to be no correlation between motor coordination and muscle strength in the offspring of vitamin E-deficient female rats.

4. Oral dose of potassium chloride, 10 mg./Kg., appears to temporarily improve motor coordination in the strain-129 dystrophic mouse (dystrophia muscularis).

5. There appears to be no correlation between weights, motor coordination, and muscle strength in the strain-129 dystrophic mouse (dystrophia muscularis).

6. There was no statistical difference between the life spans of the control group and the drug-treated groups of strain-129 dystrophic mice (dystrophia muscularis).

7. Potassium chloride (1:1500) and ephedrine sulfate (1:1000) increased the amplitude of contractions in the partially fatigued diaphragm muscle of the strain-129 dystrophic mouse (dystrophia muscularis).

8. None of the test compounds appears to significantly delay or arrest the onset of experimental muscular dystrophy.

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Gas Chromatography of Alkaloids, Alkaloidal Salts, and Derivatives

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A number of alkaloids and alkaloidal derivatives have been gas chromatographed on columns of silicone rubber SE-30. The method has also been applied to alkaloidal extracts of crude drugs. Phenolic alkaloids are readily gas chromatographed as the trimethylsilyl ethers. Alkaloidal salts have been gas chromatographed directly. The salt dissociates in the flash heater and the alkaloid is eluted as the base. Under certain conditions, many alkaloids decompose when subjected to gas chromatography. These decompositions often appear to be catalyzed by the glass wool used on top of the column packing.

In 1958 Quin used gas-liquid chromatography to study the alkaloidal composition of tobacco smoke (1–3). Two years later, Lloyd, *et al.*, demonstrated that a large number of high molecular weight alkaloids could be successfully gas chromatographed (4). This furnished a new and useful tool for research in an area in which pharmaceutical chemists have been interested since the days of Sertürner, more than 150 years ago.

This paper reports the gas chromatography of several alkaloids and alkaloidal derivatives, many of which have not been analyzed previously by this method. The gas chromatographic technique has also been applied to ex-

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tracts of alkaloidal crude drugs and to direct gas chromatography of alkaloidal salts without prior isolation of the base.

EXPERIMENTAL

A Barber-Colman model 15 gas chromatograph equipped with an argon β-ionization detector was used for the experimental work. The columns were glass U-tubes 4 to 8 feet in length and having an inner diameter of 3 mm. The solid support was Gas-Chrom P, 100–140 mesh, washed with concentrated hydrochloric acid and methanolic potassium hydroxide and treated with hexamethyldisilazane (5). The siliconized material was immediately coated with 0.1% polyethylene glycol 9000 and, after drying, with about 1% silicone rubber SE-30. Both stationary phases were applied by means of solutions in toluene as described by Horning, *et al.* (6). The treatment with polyethylene glycol was found to reduce the adsorptive effects of the support material to an appreciable extent (5). The alkaloids were introduced with a Hamilton microliter syringe as 1.0 μl. of a 0.5 to 1.0% solution in tetrahydrofuran, acetone, or chloroform. Addition of methanol was sometimes used to dissolve alkaloidal salts.

Most extracts of crude drugs were prepared by conventional methods and purified by immiscible solvent extraction. Opium, however, was extracted by treatment with a cationic exchange resin (7).

RESULTS AND DISCUSSION

The retention times of alkaloidal bases, salts, and derivatives are listed in Table I.

Alkaloidal Salts.—The alkaloids could be gas chromatographed directly as salts without prior liberation of the base. This was especially true if the acid component was an organic acid or a hydrogen halide. However, even sulfates, phosphates, and nitrates could be gas chromatographed directly. The salts dissociated in the flash heater, which was maintained at about 325°, and the alkaloids were eluted as the free bases. Therefore, no difference

TABLE I.—RETENTION TIMES IN MINUTES OF ALKALOIDS AND ALKALOIDAL DERIVATIVES

Compound	Column Temp. 175°[a]	200°[b]	225°[c]
Apoatropine	5.2	...	...
Apomorphine	...	13.0	4.6
Apomorphine hydrochloride	...	...	4.6
Atropine	8.3	...	...
Atropine sulfate	5.2 8.3	...	...
Brucine	...	...	36.8
Brucine sulfate	...	...	36.8
Caffeine	2.4	...	...
Caffeine citrated	2.4	...	...
Caffeine hydrobromide	2.4	...	...
Caffeine hydrochloride	2.4	...	...
Cinchonidine	...	8.4	3.6
Cinchonine	...	8.2	3.5
Cinchonine sulfate	...	...	3.5
Cocaine	8.6	2.6	...
Cocaine hydrochloride	8.6	2.6	...
Codeine	16.4	4.2	1.7
Codeine hydrochloride	...	...	1.7
Codeine phosphate	...	...	1.7
Cotarnine	8.0[d]	...	...
Cryptopine	...	26.0	8.6
Cryptopine oxalate	...	...	8.6
Diacetylmorphine	40.2	8.7	3.5
Diacetylmorphine hydrochloride	...	...	3.5
Dihydrocodeinone	18.3	5.0	2.3
Dihydrocodeinone bitartrate	...	...	2.3
Dihydromorphine	23.4	5.6	2.4
Dihydromorphinone	27.5	6.2	2.9
Dihydromorphinone hydrochloride	...	...	2.9
Emetine	...	...	75.0
Ethylmorphine	17.7	4.7	2.2
Ethylmorphine hydrochloride	...	...	2.2
Eucaine	2.1	...	...
Eucaine hydrochloride	2.1	...	...
Gelseminine	...	...	4.6
Gelseminine sulfate	...	...	4.6
Homatropine	5.0	...	...
Homatropine hydrobromide	5.0	...	...
Hydrastine	...	...	10.1
Hydrastine hydrochloride	...	...	10.1
Hydrastinine	3.7[d]	...	...
Hydrastinine hydrochloride	3.7[d]	...	...
Laudanosine	...	10.0	3.8
3,O-Monoacetylmorphine	24.0	6.0	2.7
6,O-Monoacetylmorphine	29.6	6.8	2.9
Morphine	27.2	6.0	2.8
Morphine sulfate	...	...	2.8
Nalorphine	41.0	8.9	3.6
Noscapine	...	45.6	13.7
Neopine	17.5	4.4	2.0
Neopine hydrobromide	...	...	2.0
Papaverine	...	17.4	6.1
Papaverine hydrochloride	...	...	6.1
Physostigmine	8.8[d]	...	...
Physostigmine salicylate	8.8[d]	...	...
Pilocarpine	6.5	...	...
Pilocarpine hydrochloride	6.5	...	...
Quinidine	...	...	5.8
Quinidine sulfate	...	...	5.8
Quinine ethylcarbonate	...	19.1	6.5
Quinine	...	15.7	5.8
Quinine hydrochloride	...	...	5.8
Scopolamine	13.7	...	...
Scopolamine hydrobromide	13.7	...	...
Sparteine	2.1	...	...
Sparteine sulfate	2.1	...	...
Strychnine	...	34.0	11.7
Strychnine nitrate	...	...	11.7
Thebaine	23.6	5.7	2.5

Column, 6 ft. long, 3 mm. I.D.; packing, 1.15% silicone rubber SE-30 on Gas-Chrom P, 100–140 mesh. [a] Inlet pressure, 20 lbs. p.s.i., flow rate, 25 ml./min. [b] 22 lbs., 25 ml./min. [c] 24 lbs., 25 ml./min. [d] Major peak; decomposition resulted in additional minor peaks.

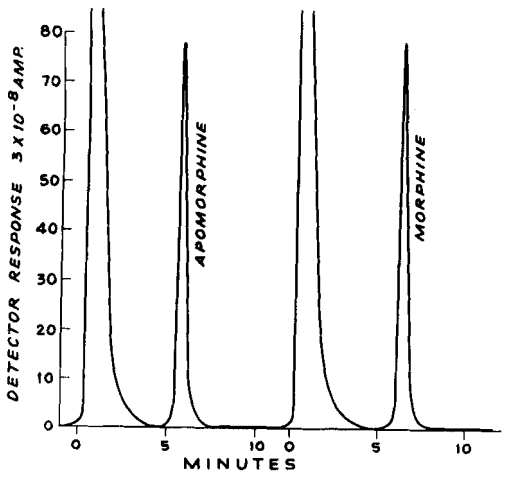

Fig. 1.—Gas chromatograms of apomorphine and morphine as the trimethylsilyl ethers. Column, 4 feet long and 3 mm. I.D.; packing, 1.2% silicone rubber SE-30 on Gas-Chrom P, 100–140 mesh; temp., 200°; inlet pressure, 20 lbs. p.s.i.

in retention time could be observed whether the alkaloid was applied as the salt or the base, Table I. Atropine sulfate produced two peaks, one of which appeared to be apoatropine resulting from dehydration in the flash heater. Although no difficulties were encountered during this work, the liberated mineral acids may in the long run have an adverse effect on the column and/or the detector.

Phenolic Alkaloids.—Phenolic alkaloids are often difficult to gas chromatograph because of adsorptive effects. Morphine generally gave only about one-half the peak height which one would expect on the basis of the amount applied. Apomorphine with two phenolic hydroxyl groups also produced a badly tailing peak. Such peaks are unsuitable for quantitative work. This difficulty was overcome by converting the phenols to the trimethylsilyl ethers (8). To a solution of 50 mg. of alkaloid in 3 ml. of tetrahydrofuran was added 2 ml. of hexamethyldisilazane. The mixture was allowed to stand in a well-closed container for about 24 hours and then gas chromatographed directly without isolation of the reaction product. Gas chromatograms of the trimethylsilyl ethers of morphine and apomorphine are illustrated in Fig. 1.

Extracts of Crude Drugs.—Figures 2 to 5 represent gas chromatograms of alkaloids extracted from several crude drugs. Where the alkaloidal mixture spans a wide range of vapor pressures, temperature programing is often useful to speed up the elution and obtain the high boiling components as relatively sharp peaks, Fig. 5. The gas chromatographic method may be used for identification of crude drugs, alkaloidal extracts and tinctures, and to differentiate between varieties of crude drugs having somewhat different alkaloidal composition. It also lends itself to a study of the various factors which may be expected to influence alkaloid production in plants.

Decompositions.—Some alkaloids could not be gas chromatographed without decomposition. Physostigmine, for example, gave two to four peaks, depending on the temperature of the flash heater. All attempts to produce a single-peak gas chromatogram of physostigmine were in vain. Hydrastinine and cotarnine also gave complex chromatograms containing several peaks. Atropine and scopolamine, which normally produced sharp, single-peak chromatograms, would under certain

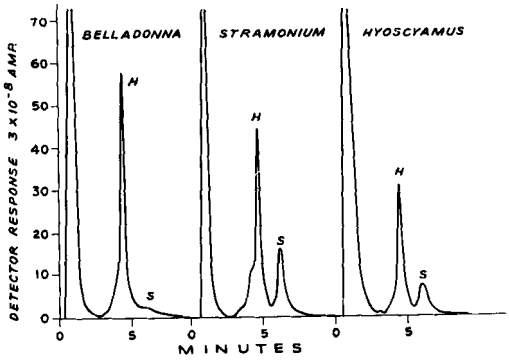

Fig. 2.—Gas chromatograms of extracts of the leaves of belladonna, stramonium, and hyoscyamus on silicone rubber SE-30. H, hyoscyamine; S, scopolamine. Column, 6 feet long and 3 mm. I.D.; temp. 200°.

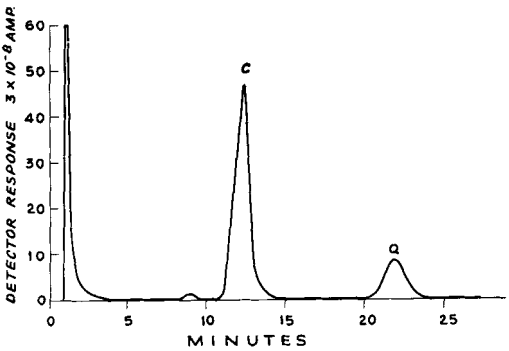

Fig. 3.—Gas chromatogram of an extract of cinchona bark on silicone rubber SE-30, 1.15%. C, cinchonine + cinchonidine; Q, quinine + quinidine. Column, 8 feet long and 3 mm. I.D.; temp. 200°.

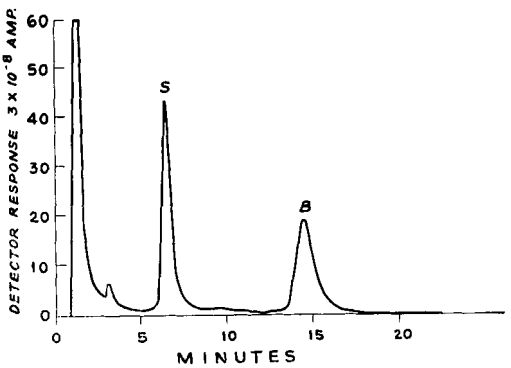

Fig. 4.—Gas chromatogram of an extract of nux vomica on silicone rubber SE-30, 1.15%. S, strychnine; B, brucine. Column, 8 feet long and 3 mm. I.D.; temp. 245°.

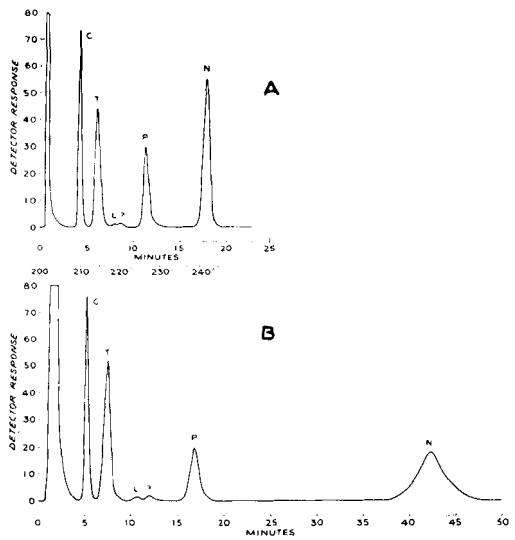

Fig. 5.—Gas chromatograms of the non-phenolic alkaloids of opium on silicone rubber SE-30, 1.15%. A, temperature programing; B, isothermal at 213°. C, codeine; T, thebaine; L, laudanosine; P, papaverine; N, noscapine. Column, 8 feet long and 3 mm. I.D.

operating conditions give two or even three peaks each. One of these appeared to represent the dehydrated alkaloid because the retention value of one of the peaks from atropine was identical with that given by apoatropine prepared according to Hesse (9). The degree of dehydration was found to be associated with the amount of glass wool placed on top of the column packing, and with the temperature of the flash heater. When a fairly large amount of glass wool was used, the degree of decomposition decreased as the flash heater temperature was reduced, but could never be entirely eliminated. When the amount of glass wool was reduced to a very small amount or removed completely, no decomposition took place even at flash heater temperatures of 350°. Similar dehydration of certain steroids has been described as a function of the flash heater temperature by Horning, *et al.* (10).

Hydrolysis and transesterification reactions were also sometimes noticed. Diacetylmorphine was eluted as a sharp peak when chromatographed alone. In mixtures with codeine, morphine or other alcoholic or phenolic substances, reactions taking place in the flash heater gave rise to several new esters not present in the original solution. Both 3, O-monoacetylmorphine (11) and 6, O-monoacetylmorphine[1] gave single peak chromatograms in the absence of glass wool. An apparent catalytic effect of glass wool resulted in peaks corresponding to morphine, monoacetyl- and diacetylmorphine.

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Antiradiation Compounds III

N-2-Mercaptoethylpiperazines

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For the purpose of obtaining 2-mercaptoethylamine derivatives having an additional basic function located two carbons distant from the nitrogen, several N-2-mercaptoethylpiperazines have been synthesized. Procedures were employed that involved conversion of the 2-hydroxyethylpiperazines to the corresponding bromo compounds with subsequent thiolation, as well as those employing ethylene sulfide. Both 1-(2′-mercaptoethyl)piperazine and 1,4-bis(2′-mercaptoethyl)piperazine were found to give some protection to mice exposed to 575 roentgens of X-irradiation.

MERCAPTANS have been recognized as effective protective agents against the lethal effects of ionizing radiation, but all of the significantly successful compounds of this variety have so far had a basic function located two or three carbons distant from the sulfhydryl group. The most outstanding examples of the mercaptoamines are 2-mercaptoethylamine (1) and 2-mercaptoethylguanidine (2). Some question remains regarding the basicity of 2-acylthioethylamines, however, which were found to have appreciable antiradiation effects in mice (3), since the acyl groups shift to the nitrogen in neutral or alkaline media (3, 4). Otherwise, a fairly strongly basic group has been required in mercaptans for antiradiation properties, so it appeared desirable to prepare mercaptoamines having an additional basic group located two carbons from the parent amine.

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