PAPER CHROMATOGRAPHIC SEPARATION OF CATECHOL AMINES USING VARIOUS SOLVENTS

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With the development of the trihydroxyindole method and the ethylenediamine condensation method, the chemical estimation of trace amounts of epinephrine (E) and norepinephrine (NE) became possible.[1-3] These methods, however, are not specific for E and NE. It has been previously reported that other catechol amines and some of the phenols produce a reaction with these methods.[4,5]

James was the first to show that E and NE could be separated by paper chromatography.[6] After his initial work, this procedure was widely used in the determination of catechol amines.[7-10] This is the most specific procedure for catechol amines but it is not sensitive enough to measure trace amounts. Since James' first study, several solvents have been employed for chromatographic separation of E and NE.[10,11] These solvents, however, are not found satisfactory by the author in the separation of other catechol amines. The purpose of the present study is to establish the most reliable solvent in the separation of catechol amines by paper chromatography, and then to analyze adrenal glands and pheochromocytoma tissues.

Methods and Material

Known amounts of E, NE, dopa, dopamine, and isoprenaline were disolved in tenth-normal HCL to be used as standard solutions. N-butanol-acetic acid-water (4:1:5 v/v); n-propanol saturated with n-HCL; phenol-acetic acid-water (50:2:50 w/v); and cresol-acetic acid-water (50:2:50 w/v), were employed as solvents. The n-butanol-acetic acid-water mixture was prepared as described by James and Kilbey.[11] Phenol-acetic acid-water and cresol-acetic acid-water solutions were prepared first by shaking the mixtures and then allowing them to stand for two hours. Their organic layers were used for chromatography.

Five adrenal glands were taken from five normal dogs. Two adrenal glands with pheochromocytomas, and one adrenal gland with cortical adenoma were removed from humans by surgery. The glands were deep-frozen imme-

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diately and stored at -20 C. The frozen glands were cut into small pieces, packed in ice, and ground in a Tien Brock grinder, and extracted several times with 0.1 n-HCL (10 ml/Gm of tissue). The extract was then centrifuged, the clear, supernatant fluid removed, and the pH adjusted to 8 by the addition of 2 n-NaOH. Extraction from solution was effected by adsorption on activat d alumina, followed by elution with 0.1 n-HCL. The eluate was dried by evaporation in a vacuum at 50 C. The dried residue was extracted in two ml of ethanol containing 1 per cent HCL. Insoluble material was removed by centrifugation and the yellow, faintly opalescent solution was evaporated to a small volume. The concentrated extracts were transferred to acid-washed Whatman No. 1 filter paper. Cresol-acetic acid-water was used as a solvent and descending chromatograms were run in an atmosphere of CO_2. After developing the chromatograms, the cresol was removed from the paper by washing it with benzene. The dried papers were then sprayed with 0.44 % K_3 Fe $(CN)_6$, dissolved in fifth-molar sodium phosphate buffer to give a pH of 7.0. The papers were then inspected under ultra-violet light.

Results

E and NE were separated completely when butanol-acetic acid-water solution was used as the solvent. Their R_f values were found to be 0.25 and 0.32, respectively (Table 1). The difference between the R_f values is great

- Table 1 -

Rf values of catecholamines in different solvents.

Substances	But - Acetic acid Rf.	Prop.-HCl Rf	Phenol - Acetic acid - Water Rf	Cresol - Acetic acid - Water Rf
Dopa	0 22	0.18	0.25	0.05
Norepinephrine	0. 25	0.13	0.28	0.10
Dopamin	0 28	0 23	0.38	0 22
Epinephrine	0 32	0 20	0 47	0 30
Isoprenaline	–	–	0.62	0.50

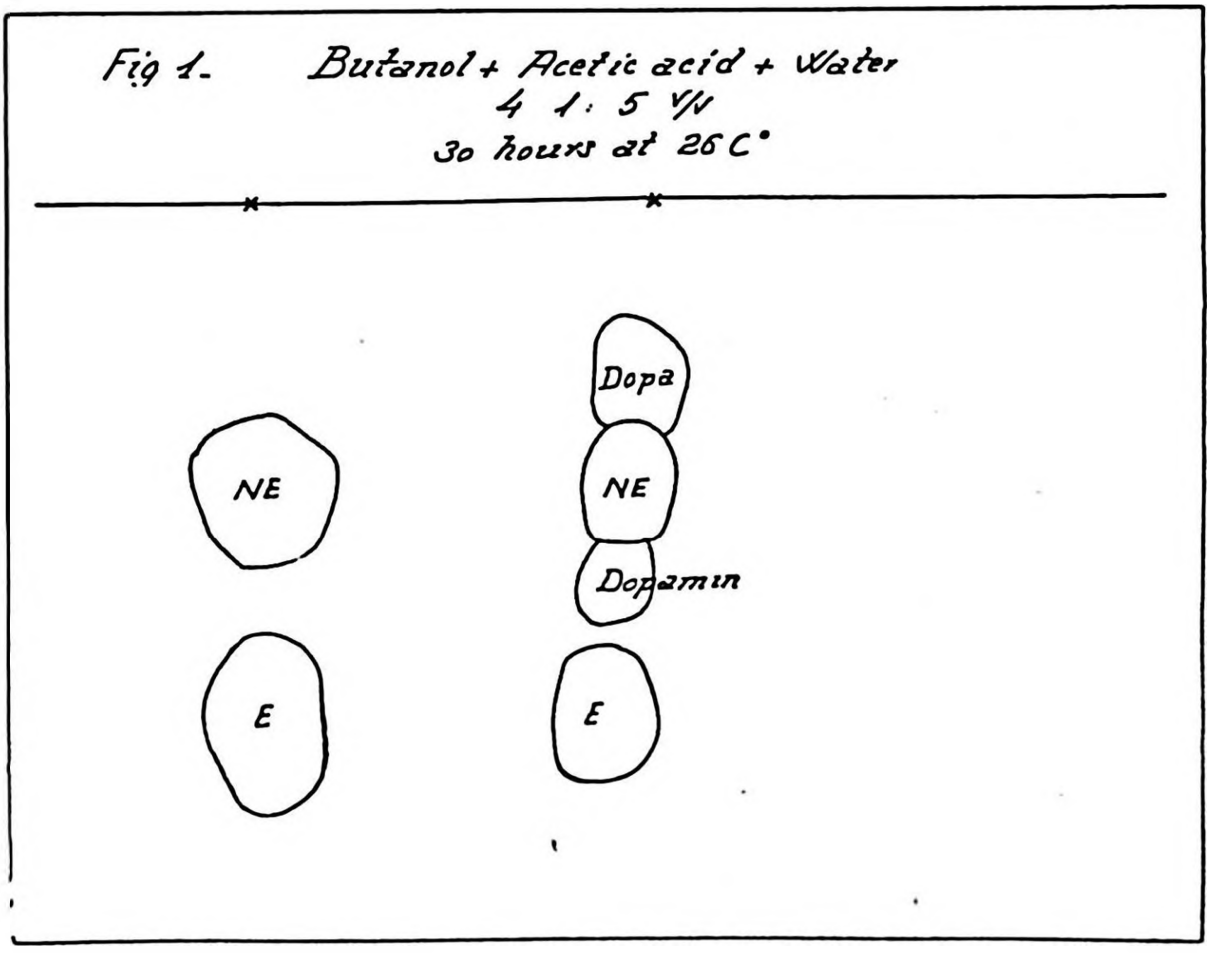
enough to separate them distinctly. The separation of other catechols was not satisfactory because their R_f value differences were not great enough to separate them (Figure 1).

When catechol amines were chromatographed using propanol saturated with HCL their separation was as poor as that obtained by using butanol-acetic acid (Figure 2).

Greater speed, allowing a more rapid separation of catechol amines, was obtained when phenol-acetic acid was used as the solvent. All of the catechol amines, except dopa and NE, were separated completely using this solvent (Figure 3). The R_f difference was not large enough to separate dopa and NE completely (Table 1).

All of the catechol amines, including dopa and NE, were separated completely when cresol-acetic acid was used as a solvent (Figure 4).

E and isoprenaline produced bright red spots and NE bluish red spots after spraying with K_3 Fe $(CN)_6$, as an indicator. These substances were also detected by their fluorescence under ultra-violet light. Other catechols, such as dopa and dopamin, produced no fluorescence so they could not be detected with this indicator under ultra-violet light.



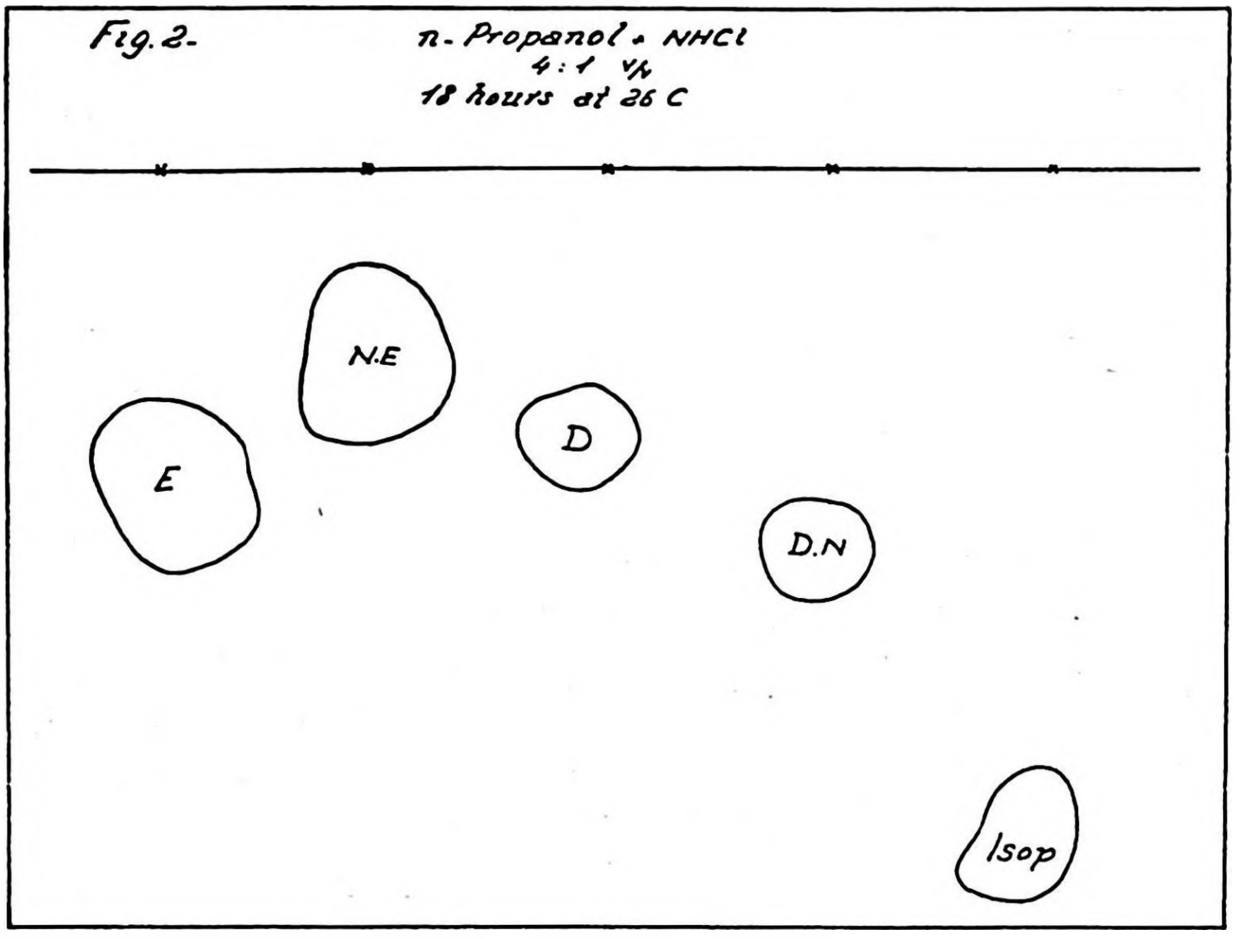


Fig. 2-
n-Propanol + NHCl
4:1 v/v
18 hours at 26 C
N.E
E
D
D.N
Isop

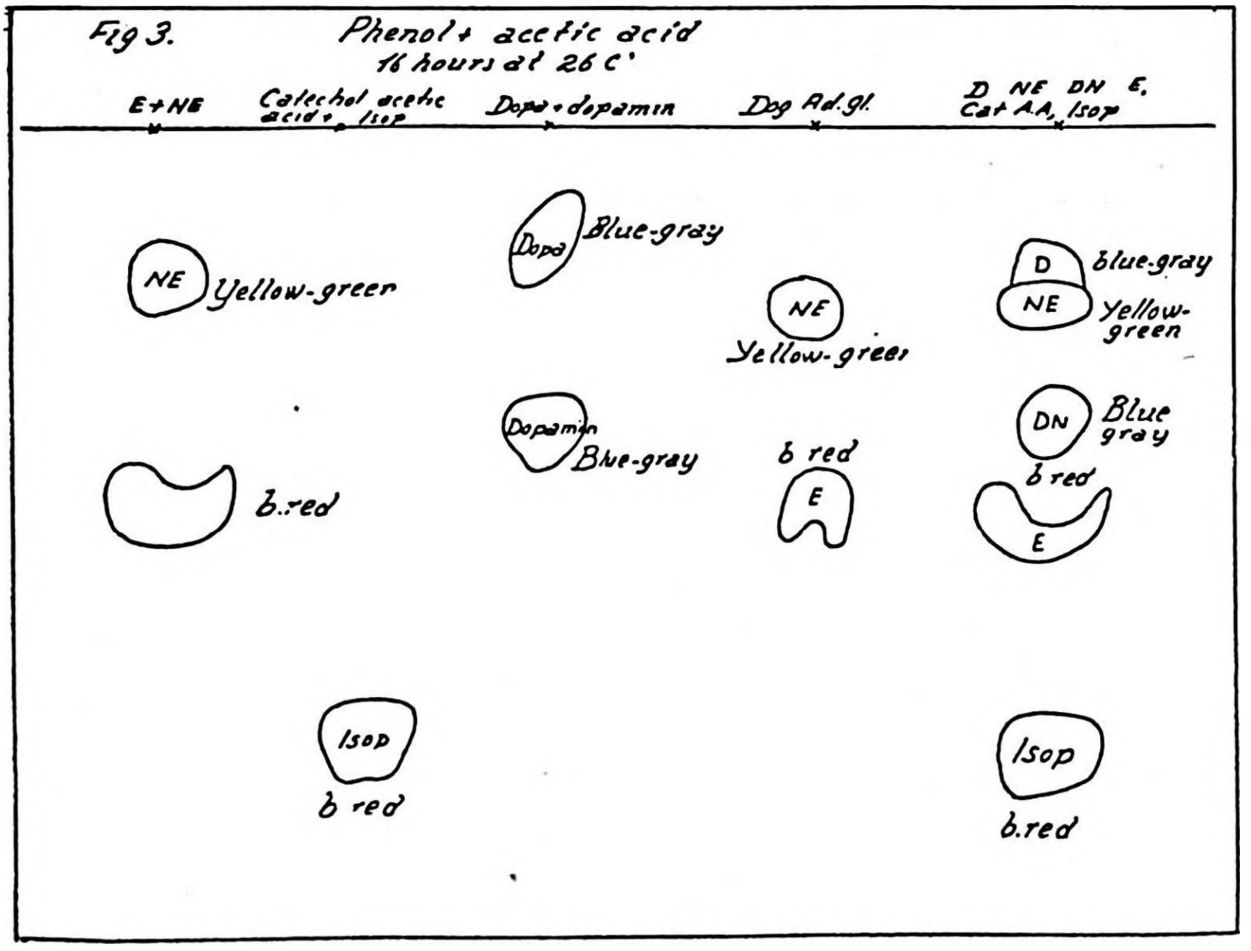
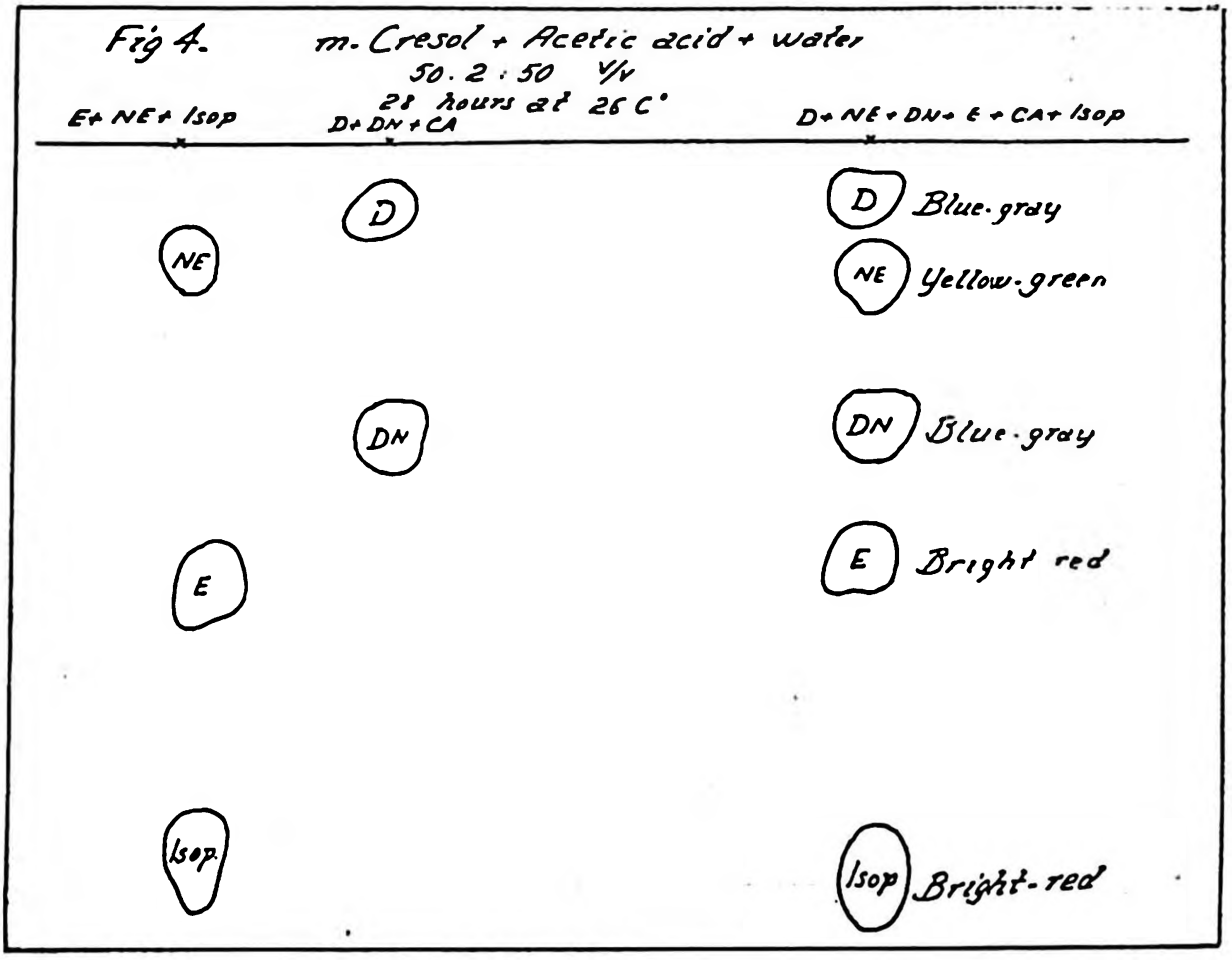


Fig 3.
Phenol + acetic acid
18 hours at 26 C°
E+NE
Catechol acetic acid + Isop
Dopa + dopamin
Dog Ad.gl.
D NE DN E.
Cat A.A. Isop
Dopa
Blue-gray
NE
Yellow-greer
NE
Yellow-greer
D
blue-gray
NE
Yellow-green
Dopamin
Blue-gray
b.red
b red
E
DN
Blue gray
b red
E
Isop
b red
Isop
b.red



When ethylenediamine was employed as a spraying solution and the paper seen under ultra-violet light, all catechol amines without exception were detected by their fluorescence. The color of the fluorescence was different for each catechol so that even incompletely separated catechols could be detected by this method (Figures 3-6). Furthermore, the sensitivity of the ethylenediamine reaction was much superior to that of K_3 Fe $(CN)_6$. With the former indicator, less than one microgram of E or NE could be detected.

Pheochromocytoma tissues, dogs' adrenal glands, and an adrenal gland with cortical adenoma were also chromatographed using cresol-acetic acid-water as a solvent. Only E and NE were found in dogs' adrenal glands and in the pheochromocytoma tissues (Figures 5 and 6). The adrenal gland with cortical adenoma produced a third substance, beside E and NE. This new substance exhibited all the properties of isoprenaline in paper chromatography (Figure 6).

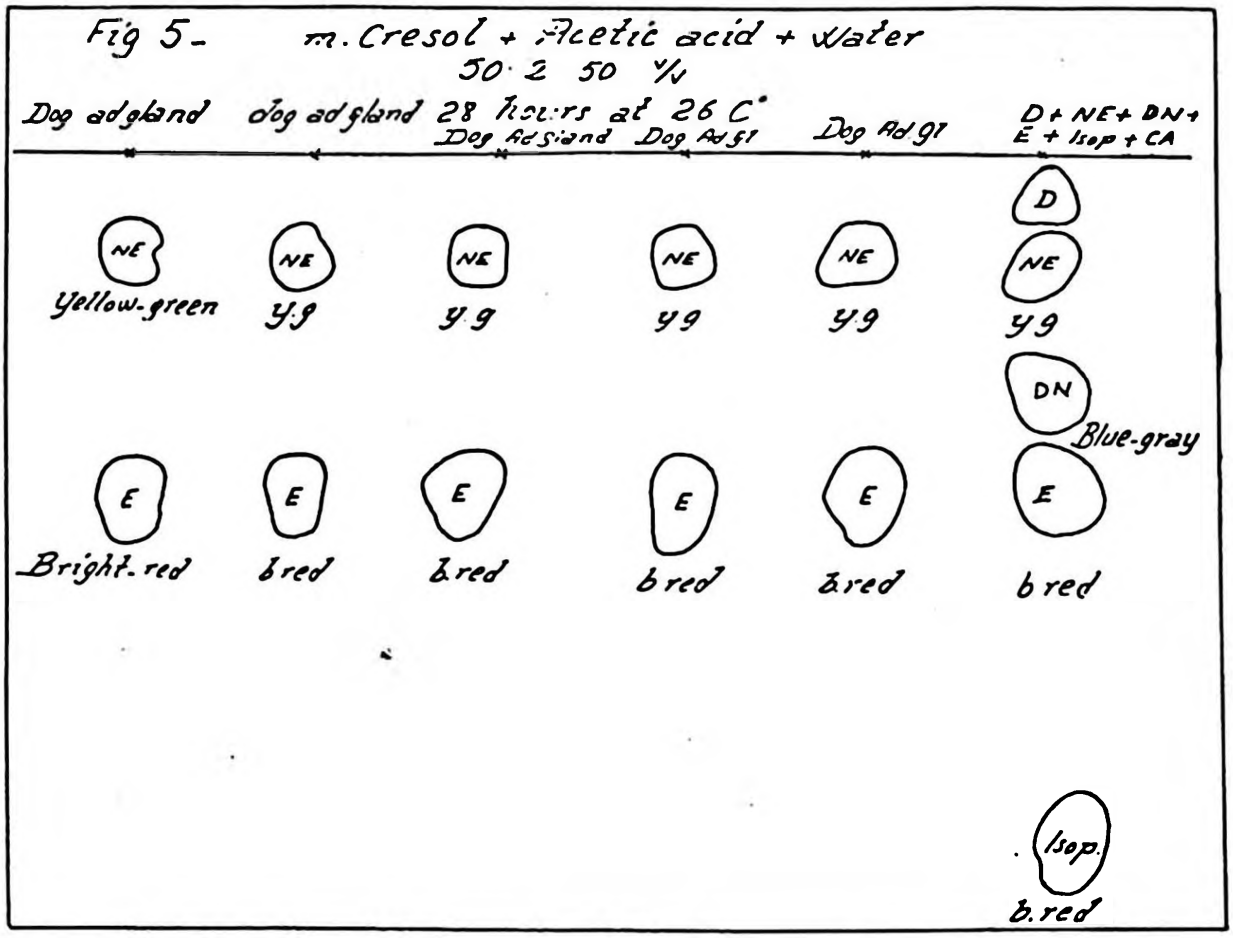


Fig 5- m. Cresol + Acetic acid + Water
50 2 50 v/v
28 hours at 26 C°
Dog adgland
Dog adgland
Dog adgland
Dog Adgl
Dog Adgl
D + NE + DN + E + Isop + CA
NE
Yellow-green
y.g
y.g
y.g
y.g
y.g
D
NE
y.g
DN
Blue-gray
E
Bright-red
b red
b red
b red
b red
b red
Isop.
b. red

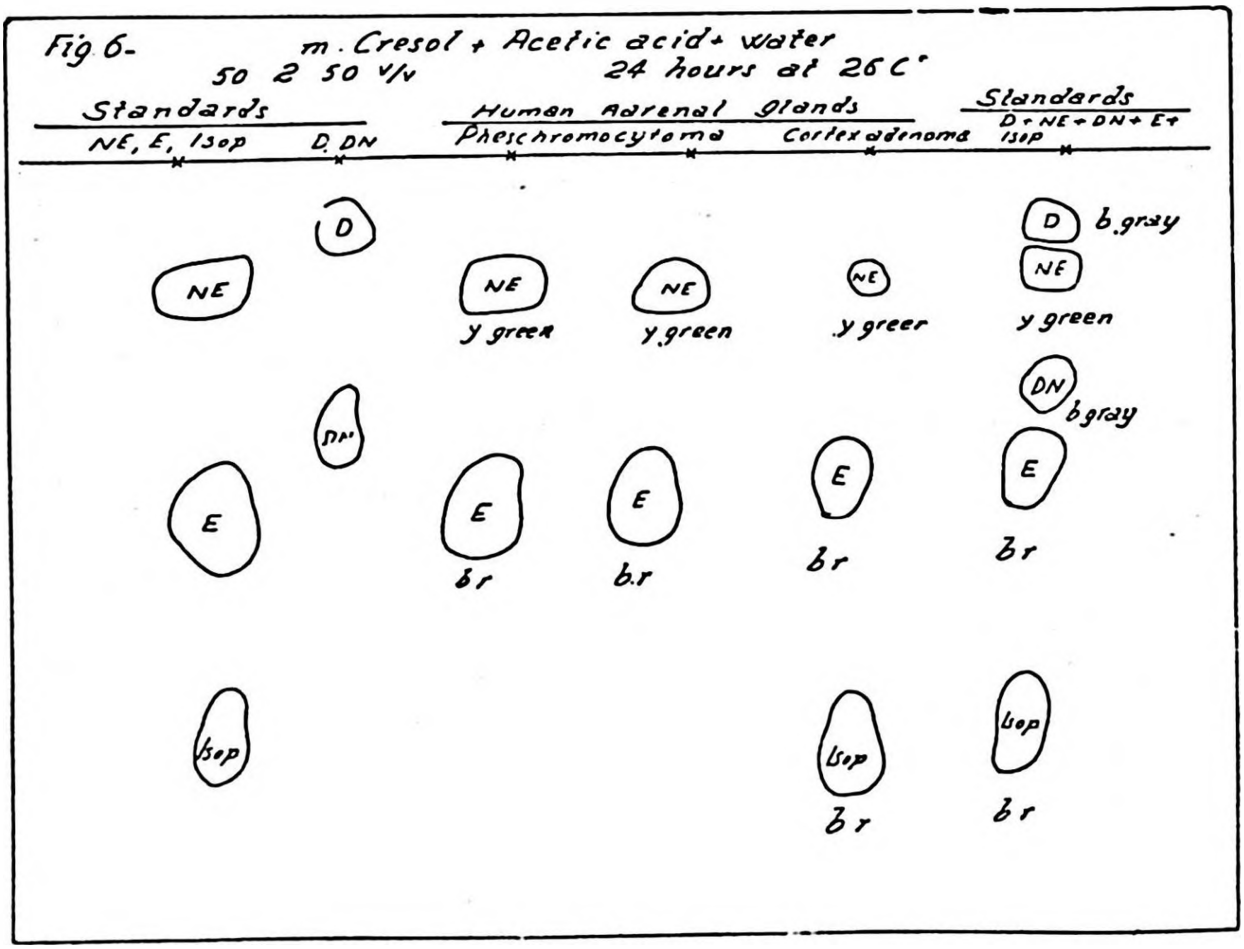


Fig 6- m. Cresol + Acetic acid + water
50 2 50 v/v
24 hours at 26 C°
Standards
NE, E, Isop
D. DN
Human Adrenal glands
Pheochromocytoma
Cortex adenoma
Standards
D + NE + DN + E + Isop
NE
D
DN
E
Isop
y green
y.green
y.green
y green
b.gray
b gray
br
b.r
br
br
br
br

Discussion

Extensive studies in the separation of catechol amines by paper chromatography have been made. For this purpose, the investigators have employed several solvents (n-butanol-water, n-butanol saturated with n-HCL, n-butanol-acetic acid-water (4:1:5 v/v), phenol saturated with water, and phenol containing 15 % w/v 0.1 n HCL). It is reported that none of the separations was as good as that produced by phenol. [11]

Using common solvents, including phenol saturated with water and phenol-HCL, the author noted that it was not possible to separate completely all of the catechols. Thus, other solvents were tried in an attempt to obtain better chromatographic separation. Phenol-acetic acid-water mixture was found to be as good as other phenol mixtures in separating NE, E, dopamine, and isoprenaline and found even better in separation of dopa and NE. The R_f difference of dopa and NE is reported by Crawford to be 0.01, using water saturated phenol, whereas this difference is 0.03 using phenol-acetic acid.

All of the catechol amines, including dopa and NE, were separated completely when cresol-acetic acid was employed as a solvent. At present, this is the most reliable solvent in the separation of catechol amines.

Ethylenediamine is much superior as an indicator to the commonly used spraying solutions, both in producing fluorescence in each catechol and in detecting small amounts of catechols.

Only E and NE are found in dogs' adrenal glands and in pheochromocytomas. A third substance, besides E and NE, was found in the adrenal gland with cortical adenoma. This third substance exhibited all the properties of isoprenaline by paper chromatography, and it may correspond to Lockett's findings in adrenal extracts of humans, monkeys and cats. [12]

Summary

In the experiments reported here, new solvents were employed to separate catechol amines by paper chromatography. Phenol-acetic acid-water and cresol-acetic acid-water mixtures found were superior to the commonly used ones.

Ethylenediamine was also found to be superior to K_3 Fe $(CN)_6$ as an indicator.

Dogs' adrenal glands, pheochromocytoma tissue, and human adrenal gland with cortical adenoma were chromatographed employing cresol-acetic

acid-water as a solvent. Only E and NE were found in the dogs' adrenal gland and in pheochromocytoma tissue. A third substance, resembling isoprenaline chromatographically, was found in the adrenal gland with cortical adenoma.

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