In the present study, tomato cuttings exposed to alcohol vapor arising from solutions of alcohol ranging from 0.1 to 95% by volume did not respond epinastically. A safer method involves the quantitative manometric method in which ethylene is trapped by mercuric perchlorate and released again with hydrochloric acid (*7*). This method may not be sufficiently sensitive for measuring very small quantities of ethylene. In such a case, it has been found satisfactory to trap the ethylene in mercuric perchlorate solution and to release it again in a closed vessel of small volume in which tomato plants are confined (*5*). The epinastic response of these plants will then be specific for ethylene if the gas arose from biological materials or did not contain other unsaturated hydrocarbons. Alternatively, the triple response can be used as a quantitative measure of the ethylene released from perchlorate solution on addition of hydrochloric acid.

References

1. CROCKER, W. *The Growth of Plants,* p. 143. New York: Reinhold, 1948.
2. KNIGHT, L. I., and CROCKER, W. *Botan. Gaz.* **55**, 337 (1913).
3. PRATT, H. K., and BIALE, J. B. *Plant Physiology* **19**, 519 (1944).
4. WILLIAMSON, C. E., and DIMOCK, A. W. In: *Plant Diseases, Yearbook of Agriculture,* p. 881. Washington, D.C.: U.S. Dept. Agr., 1953.
5. DIMOND, A. E., and WAGGONER, P. E. *Phytopathology.* In press.
6. SMOCK, R. M. *Botan. Rev.* **10**, 560 (1944).
7. YOUNG, R. E., PRATT, H. K., and BIALE, J. B. *Anal. Chem.* **24**, 551 (1952).

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Successful Transplantation of an Apparently Benign Neoplasm

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A spontaneous mammary pericanalicular fibroadenoma developed in a female albino rat of the Sprague-Dawley strain. Grossly the tumor was a hard, white, homogeneous, lobulated, encapsulated, palpable mass. Six months after complete removal, no recurrence was indicated.

As indicated in the treatise by Greenstein (*1*), some investigators consider benign tumors incapable of further growth by transplantation. Small pieces of the present tumor, 2 ml thick by 3–4 ml in diameter, were implanted subcutaneously along the milk line of albino female rats. Of the transplants, 4 of 4 in the first, 5 of 6 in the second, 6 of 8 in the third, and 3 of 4 in the fourth generation continued to grow into large tumors. Increase in the size of transplant was not evident for 4–7 wk after transplantation. If no increase was evident after 8 wk, no growth occurred later. Histology of the tumors remained the same in each generation.

Transplantations into 4 male rats and 4 female mice (Webster albino) have been unsuccessful. Transplantation by injection of macerated suspensions of the tumor have so far failed. The transplantations are being continued. Any significant change in the appearance or behavior of this tumor will be reported.

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Reference

1. GREENSTEIN, J. P. *Biochemistry of Cancer.* New York: Academic Press, 1949.

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Circular Paper Chromatography II. Isatin as a Color Reagent for Amino Acids

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In a previous publication (*1*), studies were presented of the physical factors that may influence R_f values for the circular chromatographic method (*2, 3*). Among the advantages of this method is the possibility of cutting the chromatogram into a number of segments and using various color producing reagents for each segment. Thus, it is possible to identify an individual amino acid in a band containing several other amino acids by means of *specific* color reactions. A number of such specific reagents which give colors with one or several amino acids have been mentioned in the literature (*4, 5*). This is in contradistinction to the use of ninhydrin (*6–8*) as the coloring reagent "par excellence" which gives purple or blue colors with virtually all the amino acids found in protein hydrolyzates. Exceptions to this color reaction with ninhydrin are the imino acids, proline and hydroxyproline, which give faint yellow colors. It was for this reason that Acher, Fromageot, and Jutisz (*9*) first proposed the use of isatin in *n*-butanol-acetic acid solution as a highly specific reagent which gives intense blue-green colors for proline and hydroxyproline on paper chromatograms. Their use of this reagent is based upon the experimental studies of Grassmann and Arnim (*10, 11*) on the reaction of isatin with pyrrole ring compounds to give colored products.

The chemistry of isatin is discussed in the textbook of Morton (*12*) and in a review paper by Sumpter (*13*). These sources point out that isatin reacts with both imines and amines to form products which in many cases are colored. However, only sporadic references were found to the reaction of isatin with amino acids in general (*14, 15*). Although isatin is now generally used as a specific reagent for the identification of proline and hydroxyproline on paper chromato-

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grams (*16–18*), Block (*16*) has noted that upon heating the chromatogram a number of other amino acids give color reactions with isatin. However, Block (*16*) has not stated which particular amino acids react with isatin and the limits of sensitivity of such reactions.

This paper deals with the reaction of isatin with various amino acids, the colors obtained, and the limits of sensitivity of such reactions. We have found this reagent most useful for the qualitative identification of a number of amino acids, with the circular chromatographic technique (*1*).

In the experimental work, various amounts of the amino acids listed in Table 1 were spotted on Whatman No. 1 filter paper so that each spot was 1 cm in diameter. The filter paper was dried at room temperature and then dipped into a solution of isatin (0.2% in acetone containing 4% acetic acid), dried for several minutes in air and then heated for 10 minutes at 100°. Twelve out of twenty-one amino acids tested gave distinct color reactions. It can be seen from the data in this table that a number of other amino acids besides proline and hydroxyproline give blue or blue-green colors with isatin. The approximate lower limits of sensitivity for the reacting amino acids are shown in the last column of Table 1.

TABLE 1. Reactions of various amino acids with isatin.

Amino acid*	Color	Approximate lower limits of sensitivity (μg)
1. Proline	Blue	< 1
2. Phenylalanine	Blue-green	1
3. Tyrosine	Blue-green	1
4. Tryptophan	Blue-green	1
5. Hydroxyproline	Blue-green	2
6. Glutamic acid	Lavender	2
7. Lysine	Lavender	2
8. Arginine	Lavender	2
9. Methionine	Blue-green	2
10. Histidine	Blue	2
11. Aspartic acid	Blue	5
12. Cystine	Blue-gray	5

* The following amino acids gave no appreciable color with isatin in amounts of 10 μg or less: glycine, serine, alanine, valine, threonine, leucine, isoleucine, glutamine, asparagine.

The usefulness of isatin as a color reagent for amino acids is apparent when an amino acid mixture, for example protein hydrolyzate, is run with the circular chromatographic technique employing phenol as the developing solvent (*8*) in an atmosphere of 0.1% ammonia. In this case, tyrosine-alanine and valine-methionine travel together as pairs. The presence of tyrosine and methionine, respectively, can be shown by removing a segment of the chromatogram and carrying out the isatin color reaction. The application of the isatin reaction to the identification of amino acids separated by other solvent systems has been employed extensively in this laboratory. Subsequent publications will deal with the use of this reagent for the qualitative and quantitative determination of the amino acids present in various biological fluids, for example protein hydrolyzates.

References

1. SAIFER, A., and ORESKES, I. *Anal. Chem.* **25**, 1539 (1953).
2. RUTTER, L. *Nature* **161**, 435 (1948).
3. ———. *Analyst* **75**, 37 (1950).
4. BLOCK, R. J., LESTRANGE, R., and ZWEIG, G. *Paper Chromatography, A Laboratory Manual*, p. 62. New York: Academic Press, 1952.
5. BERRY, H. K., *et al. Univ. Texas Publ. Rept.* 5109, 22 (May 1951).
6. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J. (London)* **38**, 224 (1944).
7. PRATT, J. J., and AUCLAIR, J. L. *Science* **108**, 213 (1948).
8. TOENNIES, G., and KOLB, J. J. *Anal. Chem.* **23**, 823 (1951).
9. ACHER, R., FROMAGEOT, C., and JUTISZ, M. *Biochem. et Biophys. Acta* **5**, 81 (1950).
10. GRASSMANN, W., and ARNIM, K.v. *Ann.* **509**, 288 (1934).
11. ———. *Ibid.* **519**, 192 (1935).
12. MORTON, A. A. *The Chemistry of Heterocyclic Compounds*, pp. 126–132. New York: McGraw-Hill, 1946.
13. SUMPTER, W. C. *Chem. Revs.* **34**, 393 (1944).
14. ABDERHALDEN, R. *Z. physiol. Chem.* **252**, 81 (1938).
15. LANGENBECK, W. *Ber. deut. keram. Ges.* **60B**, 930 (1927).
16. BLOCK, R. J. *Anal. Chem.* **22**, 1327 (1950).
17. MCFARREN, E. F., and MILLS, J. A. *Anal. Chem.* **24**, 650 (1952).
18. REDFIELD, R. R., and BARRON, E. S. G. *Arch. Biochem. and Biophys.* **35**, 443 (1952).

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Distemper Immunization of Ferrets by Nebulization with Egg Adapted Virus[1, 2]

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Living Newcastle disease vaccines have been administered to chickens by inhalation (*1*). The results indicate that airborne inoculation may be employed as a means of preventing the disease. The purpose of this investigation was to determine whether ferrets could be protected against virulent distemper virus (DV) through the use of this method. Ferrets were exposed to egg-adapted DV as an aerosol at definite intervals prior to a given time when all ferrets were challenged with virulent virus.

The virus suspensions for nebulization were prepared from infected chorioallantoic membranes (CAM's) of the 90th passage level of the *Onderstepoort* strain of egg-adapted DV (*2*). Seven-day-old chicken embryos were inoculated on the CAM with 0.1 ml of seed virus. After a further incubation of 7 days the CAM's were harvested and ground in a Waring Blendor with diluent to make a 10^{-1} suspension of infected membranes. The diluent was 10% horse serum in nutrient broth (Difco) with each milliliter containing 500 units of crystalline penicillin G and 500 μg of

[1] Scientific Paper No. 1263, Washington Agr. Expt. Sta.

[2] Before these studies were completed, J. A. Crawley of the University of Toronto kindly made available information which shows that mink can be protected against distemper by using the same principle as the one herein reported.

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