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.

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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

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[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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TABLE 1. The results of aerosol exposed ferrets to challenge with virulent distemper virus.

No. of ferrets in group	Date of aerosol exposure	No. of days before challenge	Day after challenge	
			Onset of signs	Death
2	8/ 9/53	32	—	—
			—	—
1	8/17/53	24	—	—
1	8/25/53	16	—	—
1	9/ 1/53	9	—	—
2	9/ 3/53	7	—	—
			—	—
2	9/ 5/53	5	—	—
			—	—
2	9/ 6/53	4	—	—
			14	20
2	9/ 7/53	3	14	Recovered
			13	21
2	9/ 8/53 (A.M.)	2	8	12
			9	12
2	9/ 8/53 (P.M.)	1.5	8	9
			8	11
2	9/ 9/53 (A.M.)	1	8	10
			9	11
2	9/ 9/53 (P.M.)	0.5	8	11
			10	12
2	9/10/53	0*	8	10
			9	11
2	9/11/53	−1	8	10
			9	11
2	Nonexposed controls		8	10
			9	11

* Simultaneous aerosol exposure and challenge virus.

dihydrostreptomycin sulfate. This dilution, which was used as the aerosol inoculum, was stored at −20° C until used. The inoculum was standardized by a titration, consisting of serial 5-fold dilutions of virus. The 50% infective dose (ID_{50}) per 0.1 ml of inoculum in chicken embryos was $10^{3.6}$.

The aerosol was produced by a DeVilbiss 40 type nebulizer operated at 3 lb pressure. The orifice of the nebulizer was fitted into a hole in one side of a chamber which measured 9 × 6 × 6 in. Approximately 0.3 ml of inoculum was nebulized into the chamber during each minute of operation. Young distemper susceptible ferrets were confined to the chamber for 3 min, the first 2 min of which the nebulizer was in operation.

The challenge virus was prepared by inoculating 2 ferrets intramuscularly with the contents of one ampoule of canine distemper vaccine (Distemperoid), Serial 174-A, which was supplied through the courtesy of Fromm Laboratories. The ferrets became moribund on the 11th day following inoculation and were sacrificed. Their spleens were removed and a 20% W/V suspension in nutrient broth was prepared. One ml of the challenge virus was given intramuscularly to each of the ferrets at intervals ranging from −1 to +32 days after exposure to the aerosol inoculum (Table 1). The appearance of conjunctivitis, nasal exudation, dermatitis, or the demonstration of DV inclusion bodies was considered the criterion of infection.

Data obtained from this preliminary experiment indicate that resistance to virulent DV can be stimulated by aerosol exposure to a living egg-adapted virus. Under the conditions of this experiment, the onset of resistance following airborne inoculation of the *Onderstepoort* strain occurred at 5 days. In a previous investigation it was shown that ferrets which were vaccinated intramuscularly with the same strain of egg-adapted virus 2 or more days prior to challenge did not develop distemper (*3*).

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Influence of Water-Soluble Vitamin E on Survival Time in Irradiated Mice[1]

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One of the current theories used in explaining the deleterious effects of ionizing radiation in animals is based upon the formation of peroxide by the action of the radiation on aqueous solutions (*1*). Mead (*2*) has shown that x-irradiation induces organic peroxide formation in linoleic acid, resulting in a chain reaction which destroys this essential fatty acid. More recently, Polister and Mead (*3*) found that *d*-γ-tocopherol was capable of protecting methyl linoleate from radiation-induced autoxidation *in vitro*. If it could be shown that this naturally occurring antioxidant was effective *in vivo*, progress would be made in radiation therapeutics. Furth, Coulter, and Howland (*4*) administered α-tocopherol in oil to rats prior to irradiation without beneficially influencing their survival time. However, Ames, Baxter, and Griffith (*5*) showed that oil-soluble vitamin E (61% α-tocopherol) decreased petechial hemorrhages of the mesentery in rats subjected to injection of radon ointment in the abdomen. These differences in results may have been due to differences in the type of irradiation used (gamma vs alpha) or to the failure of the oil-soluble material to be absorbed and become available as a tissue antioxidant. The tissue availability of the antioxidant is of greatest importance at the time of initial injury and throughout the first two postirradiation weeks if the peroxide chain reaction is to be prevented or stopped. Injections of water-soluble vitamin E would make available to the animal a natural antixodant

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[2] We wish to thank Distillation Products Industries for the water-soluble vitamin E used in this investigation.