lase in pancreas, gut and liver in the first 4 days may represent loss of an insoluble storage form of the enzyme presumed present in secretion granules. Concentration of soluble enzyme, as represented by newly synthesized enzyme just prior to molecular aggregation and storage in granules, may remain essentially unaltered under these conditions. This might account for reestablishment of diffusion equilibrium with serum as suggested by the data.

Realimentation with a complete diet after 8 days of fasting evoked an immediate rise in amylase activity in gut and liver, followed the next day by a highly significant fall in both ($p<0.01$). Realimentation had no effect on pancreas for at least 2 days and very little in 4 days. Under the stimulus of feeding, secretion of pancreatic amylase apparently kept pace with its synthesis for several days. After 16 days on non-protein diet, realimentation with complete diet was accompanied by appreciable recovery of amylase activity in gut and liver. Serum failed to depart from normal and again pancreas made very little recovery in 4 days. The gut response seems more erratic than that of other viscera. This may be associated with the presence of much muscular and other non-glandular tissue in this organ which might compete successfully for available amino acids.

Summary. Male rats were fasted for 8 days or fed non-protein diet for 16 days. Amylase activity was determined at intervals in pancreas, small gut, liver and serum. In each instance fasting caused sharp and parallel drops in amylase activity. Feeding non-protein diet 2-4 days seemed to spare amylase in small gut and serum. Liver fluctuated in the first 4 days and then remained at half normal activity for 16 days. Pancreatic amylase activity was depleted more on non-protein diet than in fasting. Feeding induces increased secretion of pancreatic amylase which, with other endogenous proteins, is probably hydrolyzed in the intestinal lumen and its amino acids absorbed. The data suggest that intestinal mucosa by its absorptive function is able to remove amino acids for protein synthesis directly from gut contents, and the liver has next choice through the portal system. Finally pancreas and other tissues are supplied by way of the peripheral circulation.

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Incorporation of DL-Lysine-2-C^{14} into Melanin Pigment of Turkey Poult Feathers. (24796)

PRAN VOHRA AND F. H. KRATZER

Dept. of Poultry Husbandry, University of California, Davis

Amino acid lysine is essential for normal feather pigmentation of turkey poults(1). Pigment in feathers is melanin in nature. It is desired to know whether lysine is a precursor for melanin synthesis in the intact poult by investigating incorporation of lysine-2-C^{14} into melanin granules of poult feathers.

Methods. Broad Breasted Bronze turkey poults of indeterminate sex, 3 to 4-day-old, were used and fed commercial poult starters. *DL-Lysine-2-C^{14} monohydrochloride.* Lysine with a specific activity of 0.3 mc/mM was obtained from Tracerlab Inc., Boston, Mass. A known weight of this sample was diluted with inactive DL-lysine hydrochloride and dissolved in water to give various solutions

with specific activities of 1.3 to 3.6 x 10^6 counts/min/ml. Four birds were injected with radioactive solutions and kept in metabolism cages(2). 0.25 ml was used for each subcutaneous injection in the abdominal area and injections were repeated every 3 hours for poults I to III. The injections were given during the day. Poult I received 3.5 ml solution and was sacrificed after 73 hours; poult II died while receiving 5th injection; and poult III died after 46 hours and had been given 2.5 ml of lysine solution. Two injections of 1 ml each of lysine solution were given in the first 23 hours to poult IV and it was sacrificed 26 hours afterwards. Flight feathers were collected from the 4 poults. *Counting procedures.* The solution or suspension of samples to be counted was plated on polyethylene planchets (2 cm in diameter) and evaporated under heat lamps to obtain samples weighing less than 10 mg spread evenly on each planchet. Samples were counted in a windowless, gas-flow counter and no correction was made for self absorption.

Melanin was removed from flight feathers of each poult separately by method essentially the same as used by Gortner(3). The feathers (about 3 g) of a poult were refluxed with 30 ml of 1*N* NaOH for 8 hrs. This was repeated once more, at which time most of the feathers were in solution. Combined NaOH solutions were filtered and acidified to pH 2 with conc. HCl. A flocculent precipitate of melanin appeared and was centrifuged off. The separated melanins were resuspended in 1*N* HCl and recentrifuged. Another washing was given with distilled water. Melanin was dissolved in 0.05*N* NaOH, filtered and reprecipitated with conc. HCl. This procedure was repeated until melanin with a constant radioactivity was obtained after 5th precipitation. The mother liquor from last precipitation was also plated and scanned for radioactivity. Melanin was dissolved in 50% NH_4OH, plated and dried on planchets. Weights of the melanin on the planchets and their radioactivity were determined. *Hydrolysis of melanin with 6N HCl.* A pooled sample (9 mg) of melanin of known activity was autoclaved in a sealed tube with 10 ml of 6*N* HCl at 15 lb pressure for 3 hrs. The melanin precipitate did not change its physical appearance but the acid solution was slightly colored. The extract was filtered from melanin aggregates which were washed first with water, then with alcohol. The extract and washings, after evaporation on a planchet for counting, left a trace of brownish layer spread evenly over entire surface. The melanin left behind after hydrolysis was only slightly soluble in 0.05*N* NaOH or 50% NH_4OH. Melanin was suspended in NH_4OH, dried on planchet and counted. The residue from the acid extract, after counting, was spotted on a Whatman filter paper No. 4 and subjected to 2 dimensional chromatographic analysis. Phenol-water (80:20) and 2, 6-lutedine-water (65:35) were used as solvents for an ascending system and spots were developed with ninhydrin.

Results. Feathers of all poults injected with DL-lysine-2-C^{14} were radioactive and did not open out of the sheaths in a normal way, probably due to high humidity in the chamber. These were washed under running water, dried and radioautographed using No-screen X-ray film for 11 days. The radioautographs showed that radioactivity was distributed throughout the feathers, however, the lower portion of shaft appeared to be more radioactive than the rest of the feathers.

Melanin isolated from feathers of all 4 poults was radioactive and no loss of radioactivity was noticed by repeated precipitations. Mother liquors from the 7th precipitation were inactive. The success in precipitating melanin to constant activity (Table I) indicated that radioactivity must be associated with melanin itself. The melanins have not been chemically defined as yet. A solution of melanin in 0.01*N* NaOH showed no characteristic absorption bands in ultraviolet or visible region. Greenstein *et al.*(4) found that melanin isolated from tissues was associated with pseudoglobulin. Hydrolysis of the conjugated proteins by pancreatic digestion to obtain melanin, still yielded protein-bound pigment. This protein was globulin in nature. Serra(5) also obtained confirmatory evidence for association of pigment (termed

TABLE I. Specific Activities of Isolated Melanins.

	No. of precipitations	Wt of melanin on planchets, mg	Time for 4096 counts, min.	Total counts/min.	Counts/min. corrected for background	Total counts/mg melanin
Background			205.95	20.0		
Poult I	5	3.6	30.35	134.0	114.0	32*
I	7	2.0	49.60	82.5	62.5	31
Mother liquor (7th p'p'tion)			196.44	20.4	.4	
Poult II	5	2.9	58.09	70.5	50.5	17
III	5	3.4	27.92	147.0	127.0	37
III	7	4.9	20.60	198.0	178.0	36
Mother liquor (7th p'p'tion)			204.58	20.0	.0	
Poult IV	5	6.8	36.55	112.0	92.0	14
Combined		5.8	26.88	152.0	132.0	23
Melanin residue after hydrolysis		6.3	52.90	77.5	57.5†	9
Acid extract			51.09	80.0	60.0†	

* Rounded figures.

† Theoretically 6.3 mg of combined melanin sample will account for 143 counts/min. Actual recovery is only 118 counts/min. or about 82%.

melanoid) with protein in melanin. Again, in contrast to the fibrous nature of the keratin protein, melanin protein was a pseudoglobulin.

It was of interest to determine if the radioactivity of melanin was due to presence of lysine-2-C^{14} in the conjugated protein. Hydrolysis of melanin with 6*N* HCl was carried out to free melanin from the protein fraction. There was no change in appearance of the black melanin aggregates after hydrolysis but they became almost insoluble in 0.05*N* NaOH, similar to the behavior noted by Serra(5). The melanin residue as well as HCl extract were equally radioactive (Table I). The residue can no longer be termed melanin because of its solubility behavior.

The acid extract was subjected to a 2 dimensional chromatographic analysis and most of the known amino acids could be detected on the chromatogram. The results indicate that melanin, as isolated from feathers, is in close association with a protein which gives melanin its characteristic solubility behavior. If this protein is partially or completely removed from melanin, its solubility in alkali is reduced. Some of the melanin residue became soluble when boiled with 1*N* NaOH but never dissolved completely as had been found by Serra(5). If the chromogen part of melanin is derived only from tyrosine, then activity of the melanin residue will have to be explained on the basis of incomplete hydrolysis of the conjugated protein or adsorption of some radioactive amino acid on the residue. Melanins are known to behave as cation exchange substances(6). But a deficiency of phenylalanine and tyrosine caused no depigmentation in poult feathers(unpublished). The present results suggest the possibility that the chromogen may be derived from lysine or a moiety of it.

A sample of feathers was hydrolyzed for 24 hrs with 6*N* HCl, and amino acids were separated by procedure of Stein and Moore(7) and the fractions plated on polyethylene planchets and scanned for radioactivity. The main activity peaks corresponded to aspartic acid, glutamic acid and lysine. Distribution of radioactivity in other amino acids (besides lysine) of feather keratin possibly accounts for the diffused nature of radioautographs of feathers. However, activity of the portion of feather which has already emerged before injection of lysine-2-C^{14} can only be explained on the basis of exchange of C^{14} either with amino acids or respiratory CO_2. It is hard to explain the role of lysine in pigmentation of turkey poult feathers but these results are consistent with the postulation that lysine functions in pigment formation through its necessity for protein synthesis rather than an "extra protein" function(1).

Summary. The melanin isolated from feathers of turkey poults injected with DL-lysine-2-C^{14} was radioactive. Part of the radioactivity was lost when melanin was autoclaved with 6.N HCl. With the protein removed, "melanin" was only slightly soluble in 0.05.N NaOH. Radioactivity was also distributed in aspartic acid, glutamic acid and lysine of the feather keratin.

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Hamster Kidney Cell Cultures in Laboratory Aspects of Epidemiologic Studies on Japanese B Encephalitis Virus.* (24797)

FRED H. DIERCKS† AND WILLIAM McD. HAMMON

Dept. of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pa.

Propagation of Japanese B encephalitis (JBE) virus in cultures of hamster kidney cells (HKC) has been studied fairly extensively(1,2) but these cultures have not been used in testing field materials collected during epidemiologic investigations. Therefore, an attempt was made to put this new laboratory tool to test with the expectation that it might have certain advantages over the use of mice. This report records results of comparative application of the *in vitro* and *in vivo* method for serum survey studies on distribution of JBE antibodies and to record results of comparative virus isolation attempts carried out in suckling mice and in HKC for recovery of JBE virus from field collected materials.

Methods. Preparation and maintenance of HKC cultures for routine laboratory use has been described(2). For our studies, these tube cultures were inoculated and observed for specific cytopathogenic responses in a manner analogous to procedures routinely in use with mice. Details of the mouse method have been recently reviewed(3) and the currently used procedure varied but slightly. Briefly reviewed, the neutralization procedure employed a screen test using 2 dilutions of virus with undiluted serum which allows for estimation of a serum neutralization index. A neutralization index of >1.6 logs was interpreted as positive; indices ranging between 1.1 and 1.6 logs as equivocal and <1.1 logs as negative. All sera were inactivated at 56°C for ½ hour prior to testing.‡ The serum-virus mixtures were incubated 2 hours at 37°C. The unit volume for tissue culture inoculation was 0.1 ml; for white mice 0.03 ml by the intracerebral route. The HKC culture contained 1 ml of maintenance fluid prior to inoculation. HKC cultures were observed daily for 7 days and mice were held for 14 days. Standard diluent for tissue culture virus in-

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† Walter Reed Army Inst. of Research, Washington, D.C.

‡ A neutralization procedure using inactivated serum was chosen to minimize the influence of labile "accessory factor" known to be necessary for demonstration of maximum neutralizing activity in test sera. Since comparative tests were performed at an interval of several years elimination of the labile factor was essential.