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TB8: Enzyme Levels in Birds

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INTRODUCTION

For many years enzymes were known to be essential catalysts in metabolism and were given a cursory discussion in courses in biochemistry and the animal and poultry sciences.

During the past decade there has been a new surge of research on enzymes, particularly blood enzymes. In the human medical field there has been new interest, especially in the area of diagnosis, and more recently in treatment and therapy.

To give a few examples, an elevation of glutamic oxalacetic transaminase (GOT) levels in the serum is used as a diagnostic aid to determine such conditions as a myocardial infarct, pericarditis, acute pancreatitis, and liver toxemia. Malic dehydrogenase (MDH) in the serum is used to detect infectious hepatitis, an acute human liver disorder. A table in the appendix summarizes the various enzymes and their application in human and veterinary medicine.

As the chicken is a good laboratory animal and the Department of Animal Pathology had extensive experience using the bird, it was decided to embark on a project relating to enzymes and the chicken during health and disease. This project was started in 1957, and with the aid of a National Institutes of Health grant #C-4957 in 1959 the work was accelerated. This bulletin covers some of the work which has not been published and at the same time summarizes some of the literature relating to enzyme activity levels in birds.

Avian and mammalian physiology differ in many respects. A vast amount of literature concerning enzyme levels in mammals is available. In comparison, there is relatively little information about enzymatic activities in birds. This meant that normal physiological values for enzymes in many cases had to be obtained.

Investigations of the effects of various factors on enzyme levels in chickens, accompanied by reviews of the literature, are presented in the following sections. A list of references is given in table II for additional information on the nature and distribution of the enzymes discussed.

ASSAY METHODS

The enzyme assay techniques which were employed are listed in table III. Where necessary, the methods were modified so that they

would be applicable to chicken plasma and tissues. The abbreviations for the enzymes are also given in table III.

The activity levels of serum and plasma are similar. Heparin does not inhibit the enzyme reactions and is, therefore, a suitable anticoagulant. Ethylenediaminetetraacetic acid (EDTA) may also be used as an anticoagulant for lactic dehydrogenase (LDH), MDH, and GOT assays. If hemolysis does not occur, heparinized blood samples may be left up to six hours before removing the plasma without affecting the LDH, MDH, GOT, or aldolase (ALD) values.

The isocitric dehydrogenase (ICD), cytochrome oxidase, and succinic dehydrogenase (SDH) assays had to be conducted on the same day that the samples were collected. The other enzymes, although relatively unstable at the refrigerator temperature of 7°C, could be frozen and stored. At -5°C, they were stable for varying lengths of time. Deterioration irregularly occurred after a week. At -40°C, the enzymes were stable up to two months.

Hematocrits were determined by a microtechnique[50]* Blood glucose levels were measured by a modification of the method of Hoffman[72] on an Auto-Analyzer**. Statistics were used to help analyze the data.[135]

*This and subsequent numbers refer to references, page 22.

**Manufactured by the Technicon Instruments Corporation, Chauncey, New York.

PHYSIOLOGICAL FACTORS

There is a wide range of variation in the plasma enzyme levels of individual chickens. The marked standard deviations listed in tables IV, VII, and VIII indicate the extent of the ranges. All the birds used in the research were hatched from eggs obtained from commercial flocks. The individual variation may be less in inbred lines. The heritability of plasma alkaline phosphatase (alkPH) levels has been studied by several investigators and Leghorns have been selectively bred for high alkPH levels [100, 139, 141, 155, 156] Variations in plasma alkPH levels have also been observed between breeds.[8] No significant differences, however, were detected in the plasma LDH, MDH, GOT, glutamic pyruvic transaminase (GPT), and acid phosphatase (acidPH) levels of two different breeds.[102]

The serum alkPH, LDH, and MDH levels of young chickens are higher than those of adults.[13, 91, 102, 119, 141] Plasma levels of LDH in ducks and liver levels of GOT in chicks also decline with age.[57, 131] The plasma levels of acidPH, GOT and GPT in mature and growing birds,

however, are similar.[14, 102] The research reported here was conducted mainly on chickens four to ten weeks of age. Data collected on the plasma ICD, LDH, MDH, ALD, and GOT levels of three groups of birds within this age bracket are presented in table IV. Although there is considerable variation in the values, no consistent trends were detected. Age, therefore, does not appear to appreciably affect plasma enzyme levels in growing birds. This agrees with observations previously published.[102]

The serum alkPH levels of hens are higher and more variable than those of roosters.[37, 38, 63, 139] Higher levels in laying hens than hens not in production have also been reported.[13, 142] Sex, however, did not affect the plasma GOT, GPT, LDH or MDH levels of mature chickens.[102] In these studies, insufficient data were collected to arrive at any conclusions about the effects of sex on the enzyme activities in adults. Lipemia presented a major problem in the collection of such data because it mechanically interfered with the assays. The lipemia is a normal phenomenon in laying hens and is controlled by estrogen.[138]

The plasma levels of alkPH, LDH, MDH, GOT, and ALD of the two sexes are similar in immature birds.[37, 103] Tanabe and Wilcox[142] reported higher alkPH levels in males than females during the growing period, but the differences were not always significant. In these investigations, no statistically significant differences were found between the male and female levels of acidPH, amylase, ICD, GPT, and phosphohexose isomerase (PHI) in plasma at approximately six weeks of age.

A number of studies concerning the effects of nutrition upon alkPH in chickens have been conducted.[3, 42, 75, 98, 108, 110, 140, 151] The effects of diet on GOT, GPT, acetyl cholinesterase (CHE) and acidPH have also been investigated to a limited extent.[14, 57, 140] The dietary levels of protein affect the blood citric acid levels of chicks subjected to various stresses.[67] Fasting also affects the blood alkPH and citric acid levels.[68, 139] Experiments on the effects of the removal of feed and/or water upon the plasma levels of LDH, MDH, GOT, ALD, and alkPH have been conducted. Each enzyme appeared to follow an individual trend during the three fasting conditions.[103]

Several investigators have reported on the effects of hormones on alkPH in birds.[23, 75, 82, 89, 142, 145] The administration of adrenal corticotrophic hormone also affects the blood citric acid levels in birds.[70]

Data on the effects of frequent bleeding and cold on enzyme activities in chickens are presented in the following sections.

Frequent Bleeding

In certain experiments a chicken may be bled more than once over a period of time. It, therefore, appeared to be advantageous to investigate how often a bird could be bled by cardiac puncture without affecting the plasma enzyme levels. The results of the investigations are summarized in table V. The controls had never been bled before. Statiscally significant differences in the average plasma enzyme levels were detected. These differences are not indicated in the table because they occurred not only between the various bleedings of the experimental birds and the experimental birds and controls bled on the same day, but also between the controls bled on different days. The differences appear to be due more to individual and daily variation than to the chronic loss of blood. The data in table IV tend to confirm this opinion. Marked fluctuations in the plasma alkPH levels of individual chickens have been observed by Bell.[13]

Similar observations about individual variation have been made concerning hematology in chickens.[44, 45, 51, 115] Frederickson[50] detected significant increases in the hematocrits of chickens bled three times at different time intervals. These elevations, however, did not follow consistent trends. A formula for predicting the hematocrits of successive blood samples from chickens was developed by Aramaki and Weiss.[4] The formula was valid when seven to eleven blood samples were removed from fowl within six hours. The hematocrits could not be predicted if birds were bled at greater time intervals. No significant differences in the hematocrits and buffy coats were found in seven chickens bled four times at weekly intervals. Data on the enzyme levels of these birds are in table IV.

Cold

The temperature control mechanism of the chicken is not fully developed until the juvenile plumage replaces the down feathers at approximately three weeks of age. Chickens are then more resistant to cold than mice, rats, and rabbits.[138] In rats, which have been exposed to 4 to 5°C for two to three weeks, the detectable shivering disappears, the total metabolism is 80 per cent above normal, and the body temperature remains approximately normal.[133] At environmental temperatures as low as 0 °C, the body temperature of an adult bird remains normal if it is allowed free movement.[138] The respiratory metabolism of the normal adult Blue North Holland fowl is minimal at environmental temperatures between 28° and 32°C and increases by 50 per cent at

5°C. In chicks and poorly feathered adults, hypothermia results in an elevation of 150 per cent.[126]

Data collected during three experiments on the effects of cold are presented in tables VI and VII. The controls were housed in isolation units. The birds exposed to cold were housed in an enclosed shed without supplementary heat. Light bulbs were placed so that they provided enough heat to prevent the water from freezing, but the chickens could not obtain warmth from them. The air temperatures were recorded by Tempscribes.* In the first experiment 70 four-week-old single-comb White Leghorns, in the second experiment 62 five-week-old Leghorns, and in the third experiment 54 five-week-old White Rocks were divided into three groups so that the effects of both prolonged and relatively short exposure to cold could be studied. The prolonged period was 15 days in the first experiment and 21 days in the other two experiments. The relatively short periods were 24 hours.

The subfreezing temperatures did not appear to harm the chickens. No lesions were found upon necropsy. The birds did not show any symptoms except one day when the temperature was unusually low (-3°F). Their feathers were ruffled and they huddled together. Their behavior returned to normal when the temperature rose above 0°F.

Rats which have been acclimated to cold consume greater quantities of food than normal. They show a transient loss in weight, however, which is followed by a retarded growth rate.[133] A similar phenomenon appeared to take place in the chickens. The weight gains of the birds subjected to prolonged exposure were significantly lower in all the experiments. Since the groups subjected to the short exposure periods were kept under the same conditions as the controls until the last day of each experiment, it was to be expected that their weight gains would be similar to the weight gains of the controls.

In rats, hypothermia produced depressed hematocrits, and elevated leukocyte counts and neuthrophilia.[66] Exposure to 41° to 44°F for 30 minutes did not affect the leukocytes in chickens.[32] A decreased plasma volume which was not accompanied by an increased hematocrit or blood specific gravity has also been reported in chickens subjected to cold temperatures.[125] The data in table VI show that in these experiments, the buffy coats were not affected and the hematocrits of the birds subjected to prolonged exposure were significantly increased.

Depressed blood suger levels have been demonstrated in chickens

*Manufactured by Bacharach Industrial Co., Pittsburg, 8, Pa.

exposed to cold.[138] In mammals, however, stress due to exposure to cold results in increased blood sugar levels.[47, 90] The birds subjected to cold for 24 hours showed decreased blood glucose levels while birds subjected to cold for longer periods of time showed increased blood glucose levels. The latter may be due to the elevated levels of metabolism in the acclimated chickens.

Increased food intake may enable an anmial to remain in positive nutritional balance during acclimation to cold. Two metabolic processes which are accelerated by cold are transaminations and the oxidations of two carbon molecules through the Krebs or tricarboxalic acid (TCA) cycle.[133] Elevated levels of GOT, GPT, and arginase have also been detected in rat livers.[81] Data on rat livers indicate that the dehydrogenases may be more affected by cold than the other enzymes in the TCA cycle. The MDH activity, however, was not increased as much as the other dehydrogenases. The LDH levels were not affected.[65] Hypothemia also affects the serum levels of enzymes in rats and dogs. Elevated levels of GOT, GPT, and ALD and normal levels of LDH and alkPH have been reported.[19, 66] Increased levels of citric acid occur in the blood of chicks exposed to cold.[67, 70] No references about the effects of cold on enzyme levels in birds were found. Fluctuations have been observed in the alkPH and lipase levels of the organs of fowl during the course of the year.[144]

In the experimental chickens, the plasma LDH and MDH levels were depressed by both the short and long exposure periods. Prolonged exposure did not affect the alkPH levels, but exposure for one day depressed them slightly. The plasma GOT levels did not appear to be affected to any extent. Although they were significantly depressed in one experiment after 24 hours, the results of the other two experiments did not verify this. The ALD levels were not affected.

The liver levels of LDH, MDH, and GOT were increased by the short exposure, but were normal after the prolonged exposure. The aconitase levels, on the other hand, were increased more during the longer exposure periods. The ALD levels were slightly depressed after the 24 hour exposure and not affected by the prolonged exposure. The liver alkPH and fumarase levels were not significantly affected by the stress.

There were no significant correlations between the plasma and liver enzyme levels.

DISEASE

An extensive amount of research on the effects of pathological conditions on enzyme activities in mammals has been conducted. The

measurements of the serum levels of some enzymes have proved to be useful diagnostic aids in human medicine (table I). Although several theories have been advanced, the mechanisms responsible for the altered enzyme levels are not fully understood.[2, 61, 76, 85, 97, 158]

Relatively little work has been done on the effects of avian diseases on enzymes. Alterations in the plasma and intestinal levels of alkPH and acidPH have been reported in chickens with coccidiosis.[34] Avian malaria affects the LDH activities in duck blood.[131] Elevated GOT and ALD levels have been observed in the plasma and muscle of chickens with inherited muscular dystrophy.[33, 41] Slight variations were noted between the levels of seven enzymes of the TCA cycle and citric acid in livers from normal and *Salmonella pullorum* infected chicks.[55] High blood citric acid levels were demonstrated in chicks infected with *Salmonella gallinarum*.[68] In a preliminary survey of chickens with clinical diseases, no abnormal plasma GPT levels were found and elevated plasma GOT levels were irregularly detected in birds with infectious synovitis, leukosis, or sufonamide toxicity. Newcastle disease and avian encephalomyelitis did not affect the GOT levels.[101] Research on the effects of drugs and insecticides has been conducted.[29, 83, 160] Preliminary studies indicate that there may be a relationship between ALD activities and different strains of Newcastle disease virus.[106] The effects of Newcastle disease on alkPH and acidPH have been investigated by histochemical methods.[116]

Burk, *et al.*[26] studied the metabolism of several transmissible fowl sarcomas. The tumors showed both a high anaerobic and aerobic lactic acid production, a respiratory quotient below unity, and a marked Pasteur effect (slower consumption and more efficient utilization of sugar under aerobic than anaerobic conditions). These properties are also characteristic of malignant tumors in mammals. The Rous sarcomas grown on chicks and in the chorioallantoic membrane of embryos possessed similar metabolisms except in the latter, the aerobic glycolysis was lower and the respiratory quotient and rate were higher. Further studies in Rous sarcomas have demonstrated the presence of the Crabtree effect (decrease of respiration by the addition of glucose), that lactic acid is apparently the major product of glucose metabolism, and that there is preferential oxidation of the carbon-1 of glucose.[6, 7, 92, 93, 94]

In studies on avian leukosis, Burk, *et al.*[25] did not detect any alterations in the metabolism of affected spleens and bone marrows. The metabolism of leukotic livers, however, did differ from that of normal livers. Erythroblastosis and myeloblastosis viruses have been grown in

tissue culture. Comparative studies on the two types of cells produced did not reveal any differences in the average oxygen consumption and carbon dioxide evolution. Direct measurement of the glucose and lactate concentrations in surrounding the media demonstrated an accumulation of lactate with the erythroblasts but not the myeloblasts. This indicated that the myeloblasts were able to convert the equivalent of glucose and an extra amount of lactate from the medium to carbon dioxide.[11] Low blood glucose and high blood lactate levels have been observed in birds during the late stages of myeloblastosis and erythroblastosis.[12]

The enzyme, adenosine triphosphatase(ATPase), has been associated with the virus of myeloblastosis. Relationships have been observed between the concentration of the virus in the plasma, the infectivity of the plasma, and the plasma levels of this enzyme.[10] Similar relationships have been detected between the virus and inosine triphosphatase.[60] The plasma levels of ATPase in chickens infected with erythroblastosis virus are only slightly above normal. No definite relationships have been observed between this virus and the enzyme.[22] No acidPH or alkPH activity could be found in association with myeloblastosis virus grown in tissue culture.[149] Elevated ATPase levels, low alkPH levels, and normal acidPH levels have been observed in plasma from chickens with visceral lymphomatosis.[91]

Comparisons of enzymes involved in carbohydrate metabolism in Rous sarcoma and the chorioallantoic membranes of chick embryos have been made. No glucose-6-phosphatase activity could be demonstrated. The levels of glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and fructose-1,6-diphosphatase were similar in the two tissues. The levels of phosphoglucomutase, PHI, and LDH were higher in Rous sarcoma. The elevated activities of these glycolytic enzymes correspond to the increased production of lactate in the sarcomas.[147, 148] The increases in LDH and PHI levels of the tumors grown in chorioallantoic membranes are much more marked than the increases in tumors grown in pancreases reported in table VIII. In a preliminary study, the phosphorylase level of the Rous sarcoma was low when compared with normal muscle collected from the same chicken.[114] Little acidPH activity is present in Rous tumor cells.[31, 95]

Rous Sarcoma

The Rous sarcoma virus was employed for studies on the effects of neoplasms on avian enzyme levels. Lyophilized virus was generously supplied by Dr. W. Ray Bryan at the National Institutes of Health, Bethesda, Maryland.

In a series of experiments, the virus was inoculated either subcutaneously, intramuscularly, intraperitoneally, or intracardially. The plasma levels of LDH, MDH, ICD, GOT, ALD, ATPase, and Ch-E were determined periodically during the course of the disease. At first, the enzyme levels appeared to follow definite trends. But these observations could not be verified in the later experiments. Levels beyond the ranges of the controls were found in chickens which were severely affected, chickens whose tumors had undergone complete remission, and chickens which had never develop tumors.

There was a wide range of susceptibility to the virus among the experimental birds. The percentage of chickens which were affected ranged from 100 percent of five-week-old birds inoculated with 0.2 ml of a 10^{-4} dilution to 66.7 per cent of two-week-old birds inoculated with 0.2 ml of 10^{-1} dilution. It, therefore, seemed advisable to use a more virulent virus in order to obtain a more uniform infection. Dr. Alvin R. Whitehill, Head of the Department of Bacteriology, University of Maine, generously supplied the virus used in the succeeding experiments. He had passed the Rous virus via the pancreas according to the method described by Popken and Baughn.[121]

Single comb White Leghorns were injected intraperitoneally in the vicinity of the pancreas with 0.2 ml of a 10^{-3} dilution of the virus at three weeks of age. A survey of the plasma and pancreatic enzyme levels was conducted. A total of 15 enzymes was assayed. The results are presented in table VIII. The purpose of the survey was to find an enzyme, the level of which would be sufficiently altered by the disease, to act as an aid in following the course of neoplastic growth in a live bird. Although statistically significant differences were found, none of the enzymes fulfilled this requirement. There were no significant correlations between the plasma and pancreatic levels.

SUMMARY AND CONCLUSIONS

The effects of various factors on the activities of 17 enzymes in chickens were studied. Detectable levels of 11 of these enzymes were found in avian plasma. A wide range of variation in the plasma enzyme levels exists among individual birds. This individual variation probably explains why the factors did not dramatically affect the enzyme levels.

Age had little effect on the plasma levels of aldolase, alkaline phosphatase, glutamic oxalacetic transaminase, lactic dehydrogenase, and malic dehydrogenase in growing birds four to ten-weeks-old. The plasma levels of all the enzymes in immature males and females are similar.

In order to study the effects of different stresses, chickens were subjected to frequent bleeding, cold, and inoculation with Rous sarcoma

virus. Statistically significant differences were observed and the individual enzymes appeared to follow different trends. The ranges of the plasma levels of the treated birds, however, overlapped the ranges of the controls in all the experiments. The effects of cold on liver enzyme levels and the effects of Rous sarcoma virus on pancreas enzyme levels were also investigated and similar observations were made.

The literature pertaining to the effects of these and other factors on avian enzyme activities is reviewed.

Enzyme	Location	Significant Pathology or Physiology
Amylase	serum	pancreatitis
Cholinesterase	serum	hepatitis, cirrhosis, cholangitis
Alkaline phosphatase	serum	obstructive jaundice, cholangitis, rickets, osteomalacia, osteogenic sarcoma, chrondrosarcoma, metastatic carcinoma, myeloid leukemia
Acid phosphatase	serum	mammary carcinoma, Gaucher's disease, Niemann-Pick's disease
	semen	azoospermia
Transaminases GOT	serum	myocardial infarct, pericarditis, acute pancreatitis, liver toxemia, gangrene
GPT	serum	liver infarct, acute hepatitis
Lactic acid dehydrogenase	serum	cardiac infarct, muscular dystrophy, abdominal tumors, leukemia, pernicious anemia, pregnancy
Aldolase	serum	acute hepatitis, muscular dystrophy, liver diseases, prostatic carcinoma
Uropepsin	urine	pernicious anemia, gastric ulcer, duodenal ulcer, cancer stomach (lowered), adrenal function
Beta-glucuronidase	serum	pre-eclampsia, cancer reproductive tract (female)
	cerebospinal fluid	glioblastoma multiforme
Lysozyme	gastric juice	gastric ulcers
	stool	ulcerative colitis
Ceruloplasmin	serum	psychoses-schizophrenia, tuberculosis
Phosphohexoseisomerase	serum	acute hepatitis
Malic dehydrogenase	serum	myocardial infarction, infectious hepatitis
	urine	nephritis, nephrosis
Glutamic acid dehydrogenase	white blood cells	leukemia
Ornithine transcarbamylase	serum	epidemic hepatitis, cholelithiasis
Cholesterol esterase	serum	liver disease (decreased), Kala-azar
Catalase	blood	macrocytic anemia
Peptidases	serum-urine	lobar pneumonia, liver disease, infectious mononucleosis
Lipase	urine	pancreatic carcinoma
Elastinase	serum	atherosclerosis
Congenital Enzyme Defects (recessive characteristics)	urine	imbecillitas
	phenylpyruvic acid	phenylpyruvica, alcaptonuria, cystinosis, flycinuria, galactosemia

ENZYME THERAPY

Enzyme	Disease Treated or Conditions
Hyaluronidase	to diminish viscosity to increase permeability keloids obstetrics ophthalmology kidney stones
Thrombin	to stop bleeding wounds
Trypsin	to digest necrotic tissue
Streptokinase) Streptodornase)	to liquify thick pus
Plasmin	for thrombosis
Pancreatic deoxyribonuclease	to dissolve viscous secretions (lungs)

Table II
REFERENCES TO ENZYMES IN BIRDS

Enzyme	Reference Number
Acid phosphatase	1, 14, 15, 16, 17, 29, 31, 32, 34, 49, 56, 82, 88, 91, 95, 100a, 102, 107, 116, 140, 150, 154
Aconitase	55, 127
Aldolase	33, 41, 103, 106
Alkaline phosphatase	1, 3, 5, 8, 13, 15, 16, 17, 18, 23, 24, 29, 34, 36, 37, 38, 42, 49, 53, 54, 63, 64, 71, 75, 82, 84, 87, 88, 89, 91, 98, 99, 100, 100a, 102, 103, 108, 109, 110, 111, 112, 113, 116, 117, 118, 119, 128, 137, 139, 140, 141, 142, 144, 145, 151, 152, 153, 154, 155, 156, 160
Amylase	48, 73, 104, 120, 130, 138
Cholinesterase	5, 27, 28, 83, 105, 140, 143
Cytochrome oxidase	43, 55, 69
Fumarase	55, 127
Glutamic oxalacetic transaminase	33, 35, 40, 41, 49, 57, 62, 100a, 101, 102, 103, 137, 160
Glutamic pyruvic transaminase	35, 40, 49, 100a, 101, 102, 140
Isocitric dehydrogenase	39, 49, 55, 127
Lactic dehydrogenase	5, 30, 49, 55, 77, 78, 80, 86, 96, 100a, 101, 102, 103, 131, 136, 147, 148, 160
Malic dehydrogenase	9, 49, 53, 55, 58, 100a, 101, 102, 103, 127, 136
5-Nucleotidase	56
Phosphohexose isomerase	20, 148
Succinic dehydrogenase	9, 43, 53, 54, 55, 127, 129

Table III
ENZYME ASSAY METHODS

Enzyme	Method	Expression of Activity
Acid phosphatase (acidPH)	Powell and Smith, 1954[122]	King-Armstrong (K-A) units
Aconitase	Racher, 1950[123]	One unit = Amount of enzyme activity which will cause an increase of 0.001 in optical density per minute under standard conditions
Adenosine Triphosphatase (ATPase)	Green, *et al.*, 1954[59]	Number of minutes to end point
Aldolase (ALD)	Friedman and Lapan, 1958[52]	Dihydroxyacetone units
Alkaline phosphatase (alkPH)	Powell and Smith, 1954[122]	King-Armstrong (K-A) units
Amylase	Smogyi, 1938[133, 134]	Smogyi units
Cholinesterase (acetyl) (CHE)	Huerga, *et al.*, 1952[74]	Micromoles of acetylcholine hydrolyzed per hour
Cytochrome oxidase	Umbreit, *et al.*[145, 146]	Qo_2
Fumarase	Racker, 1950[123]	Same as for aconitase
Glutamic oxalacetic transaminase (GOT)	Karmen, 1955[79]	One unit = amount of enzyme activity which will cause a decrease of 0.001 in optical density per minute under standard conditions
Glutamic pyruvic transaminase (GPT)	Reitman and Frankel, 1957[124] Sigma Tech. Bul. #505*	Sigma-Frankel (S-F) units
Isocitric dehydrogenase (ICD)	Wolfson and Williams-Ashman, 1957[156, 157]	Same as for aconitase
Lactic dehydrogenase (LDH)	Wroblewski and LaDue, 1955[158, 159]	Same as for glutamic oxalacetic transaminase
Malic dehydrogenase (MDH)	Siegel and Bing, 1956[131, 132] McDaniel and Chute, 1961[102]	Same as for glutamic oxalacetic transaminase
5-Nucleotidase	Dixon and Purdom, 1954[46]	One unit=mg. p liberated per hour
Phosphohexose isomerase (PHI)	Bodansky, 1954[21]	Bodansky units
Succinic dehydrogenase (SDH)	Umbreit, *et al.*[145, 146]	Qo_2

The plasma levels are given as units per ml except amylase, acid and alkaline phosphatase and 5-nucleotidase are given as units per 100 ml. The tissue levels are given as units per mg. dry weight except alkaline phosphatase and fumarase are given as units per gm. dry weight. The data in the tables represent the mean ± S.D.

*Sigma Chemical Co., St. Louis, Mo.

Enzyme	Method	Expression of Activity

Table IV
PLASMA ENZYME LEVELS IN YOUNG CHICKENS

Enzyme	No. birds	Age (wk)	Activity units/ml	Enzyme	No. birds	Age (wk)	Activity units/ml
Isocitric dehydrogenase	16	4	2,635 ± 560	Malic dehydrogenase	16	4	1,103 ± 74
		5	3,154 ± 1,676			5	1,322 ± 363
		7	2,010 ± 428			6	727 ± 130
		8	2,855 ± 821			7	793 ± 124
						8	1,644 ± 415
	7	7	1,365 ± 625			9	789 ± 205
		8	1,080 ± 220			10	1,276 ± 515
		9	1,422 ± 316				
		10	2,225 ± 738		7	7	657 ± 159
						9	1,043 ± 349
Lactic dehydrogenase	16	4	1,070 ± 185			10	1,097 ± 275
		5	1,324 ± 391				
		6	1,377 ± 311		20	5	1,080 ± 255
		7	1,046 ± 411			7	844 ± 178
		8	1,440 ± 839				
		9	763 ± 175	Aldolase	7	7	21 ± 4
		10	1,078 ± 325			8	27 ± 7
						9	22 ± 7
	7	7	1,108 ± 540			10	28 ± 9
		8	777 ± 165				
		9	1,125 ± 300		20	5	18 ± 5
		10	1,450 ± 428			7	25 ± 9
	20	5	1,013 ± 278				
		7	1,139 ± 355	Glutamic oxalacetic transaminase	7	7	176 ± 23
						8	170 ± 14
						9	340 ± 90
						10	339 ± 83

Definitions of enzyme activity units given in table III.

Table V

EFFECTS OF FREQUENT BLEEDING ON PLASMA ENZYME LEVELS IN CHICKENS

Enzyme	Interval between bleedings (days)	Average Plasma Levels Experimental birds Bleeding No. 1	2	3	Controls Bleeding No. 1	2	3
Aldolase	1	28	24	19	21	22	21
	2	16	19	15	21	21	19
	2	15	17	16	16	15	19
	7	14	13	24	21	14	30
Alkaline phosphatase	2	147	125	133	109	91	137
	2	73	82	99	—	—	73
Glutamic oxalacetic transaminase	1	321	161	175	159	174	202
	2	289	96	227	159	202	176
	2	159	119	104	160	94	89
	2	168	145	125	—	—	161
	7	167	182	137	159	168	112
Lactic dehydrogenase	1	964	525	776	564	643	586
	2	939	572	899	564	586	624
	2	817	926	845	855	893	895
	2	1,024	744	795	—	—	782
	7	660	563	543	564	556	512
Malic dehydrogenase	1	778	490	697	668	599	683
	2	821	608	797	668	683	766
	2	698	740	729	751	772	828
	2	720	529	528	—	—	524
	7	532	666	405	668	537	392

Definitions of enzyme activity units given in table III.

Table VI

EFFECTS OF COLD ON CHICKENS

Type of Data	Exp. No.	Controls	Chickens Exposed to Cold 15 to 21 days	24 hours
Air temperature (°F)	1	60 ± 4	35 ± 9	48 ± 4
	2	68 ± 8	32 ± 9	39 ± 5
	3	67 ± 2	25 ± 12	28 ± 5
Initial body weight (gm)	1	237.9 ± 54.5	247.9 ± 44.4	243.8 ± 39.4
	2	341.2 ± 47.4	341.6 ± 46.8	342.1 ± 44.6
	3	578.8 ± 82.3	581.1 ± 64.9	580.8 ± 68.3
Final body weight (gm)	1	563.3 ± 106.0	518.7 ± 101.9*	564.7 ± 116.8
	2	688.2 ± 109.9	603.0 ± 96.4*	698.4 ± 107.9
	3	1,338.7 ± 180.2	1,103.6 ± 198.0†	1,288.4 ± 253.0
Hematocrit (%)	1	26.6 ± 2.7	29.0 ± 4.0†	26.9 ± 2.9
	2	26.2 ± 1.6	31.6 ± 3.8†	26.0 ± 2.2
	3	25.8 ± 1.4	28.7 ± 2.6†	25.2 ± 1.8
Buffy coat (%)	1	0.40 ± 0.29	0.34 ± 0.24	0.38 ± 0.21
	2	0.27 ± 0.19	0.32 ± 0.04	0.29 ± 0.16
	3	0.37 ± 0.16	0.25 ± 0.07	0.43 ± 0.13
Blood glucose (mg%)	1	241 ± 41	269 ± 31*	187 ± 39†
	2	254 ± 19	262 ± 22	238 ± 23*
	3	242 ± 6	273 ± 35†	235 ± 28

* $P < 0.05$
† $P < 0.01$
Data expressed in mean ± S. D.

Table VII

EFFECTS OF COLD ON AVIAN ENZYME LEVELS

	Enzyme Levels (x ± S.D.)					
	Plasma			Liver		
	Exposed to Cold					Exposed to Cold
Enzyme	Controls	15 to 21 days	24 hours	Controls	15 to 21 days	24 hours
Aldolase						
Exp. No. 1	20.0± 9.0	21.5± 8.4	17.3± 4.4			
Exp. No. 2	23.9± 3.2	20.6± 2.2	22.0± 2.6	1.88± 0.27	1.86± 0.23	1.79± 0.20
Exp. No. 3	18.6± 1.7	20.2± 3.6	19.4± 2.0	1.83± 0.21	1.96± 0.27	1.65± 0.24*
Alkaline phosphatase						
Exp. No. 1	167 ± 82	182 ±109	145 ± 67	—	—	—
Exp. No. 2	184 ± 42	161 ± 64	102 ± 60	75.3 ± 18.1	63.8 ± 15.8	66.7 ± 16.2
Exp. No. 3	138 ± 75	114 ± 51	89 ± 29*	46.4 ± 12.1	58.3 ± 17.2	54.1 ± 17.6
Glutamic oxal-acetic transaminase						
Exp. No. 1	239 ± 74	242 ± 57	213 ± 69	—	—	—
Exp. No. 2	140 ± 33	135 ± 30	149 ± 28	1,295 ±301	1,148 ±374	1,445 ±178
Exp. No. 3	134 ± 26	124 ± 17	98 ± 29†	1,167 ±208	1,255 ±255	1,350 ±350*
Lactic dehydrogenase						
Exp. No. 1	1,377 ±345	1,274 ±346	1,123 ±305*	—	—	—
Exp. No. 2	842 ±209	611 ±112†	815 ±201	2,404 ±738	2,068 ±542	3,451 ±913†
Exp. No. 3	879 ±145	526 ± 64†	636 ±144†	1,892 ±387	2,032 ±529	2,159 ±526
Malic dehydrogenase						
Exp. No. 1	956 ±398	946 ±313	871 ±295	—	—	—
Exp. No. 2	570 ±134	491 ±121†	533 ±136	1,763 ±588	1,529 ±570	2,413 ±630†
Exp. No. 3	571 ±104	354 ± 46†	426 ± 79†	1,326 ±286	1,529 ±405	1,669 ±394*
Aconitase						
Exp. No. 2	—	—	—	36.3 ± 10.3	39.9 ± 17.4	37.7 ± 12.2
Exp. No. 3	—	—	—	35.3 ± 14.2	46.5 ± 17.6*	45.9 ± 12.6*
Fumarase						
Exp. No. 2	—	—	—	189.8 ± 35.7	209.3 ± 40.0	178.6 ± 17.6
Exp. No. 3	—	—	—	203.7 ± 28.1	219.3 ± 37.6	217.5 ± 44.4

* $P < 0.05$
† $P < 0.01$
Definitions of enzyme activity units given in table III.

Table VIII

ENZYME LEVELS OF CHICKENS INOCULATED WITH ROUS SARCOMA VIRUS

	Inoculated Birds						Controls		
	Postive			Negative					
Enzyme	No. birds	Plasma levels	Pancreas levels	No. birds	Plasma levels	Pancreas levels	No. birds	Plasma levels	Pancreas levels
Acid phosphatase	26	66.6±57.0*	102.7±44.3	34	92.0±79.6	86.3±28.0	47	106.2±79.5	98.0±24.1
Aconitase	33	No activity		38	No activity		48	No activity	
Aldolase	32	19.3±3.5	0.657±0.241	5	17.7±1.0†	0.945±0.409	33	21.1±2.4	0.651±0.100
Alkaline phosphatase	31	99±88†	12.7±9.0*	29	160±111*	3.6±2.7†	57	241±185	9.4±5.4
	32	184±179†	—	5	197±80†	—	33	343±255	—
Amylase	33	2,999±2,400†	2,642±2,534*	38	1,535±1,211*	2,848±2,063†	48	1,114±320	4,047±2,514
Cytochrome oxidase	12	Not done	0.61±0.03†	3	Not done	2.00±1.12*	16	Not done	3.75±1.50
Fumarase	33	No activity	57.2±22.2†	38	No activity	61.9±29.0†	48	No activity	74.3±22.5
Glutamic oxalacetic transaminase	32	212±156	56±27†	5	147±34	136±17*	33	162±27	155±24
Glutamic pyruvic transaminase	31	29.6±11.3†	2.85±1.75	29	19.8±12.5	1.72±0.88†	56	21.5±8.5	3.30±1.42
Isocitric dehydrogenase	31	56±24	78±30†	29	67±41	122±46	57	66±21	129±40
Lactic dehydrogenase	32	1,225±1,062†	347±163†	5	703±56†	243±37	33	872±175	234±83
Malic dehydrogenase	32	986±741*	190±108†	5	571±56†	282±48†	33	704±132	372±100
5-Nucleotidase	34	low levels in 6 samples	0.41±0.27	41	low levels in 8 samples	0.41±0.20	48	low levels in 2 samples	0.40±0.20
Phosphohexose isomerase	31	376±235*	52.3±29.2†	29	398±175*	41.8±26.9	56	526±240	32.6±24.1
Succinic dehydrogenase	13	Not done	0.086±.291†	3	Not done	0.450±0.309	18	Not done	0.945±0.235

* $P < 0.05$ † $P < 0.01$ Definitions of enzyme activity units given in table III.

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