$P < .005$). Following the precedent of others, all can temperatures below an arbitrary level, in this case 30°C, were ignored (Fig. 2, upper portion). The product-moment correlation coefficient obtained is $-.09$; $P < .05$. This indicates that the can temperature is independent of the air temperature. The range of correlation approximates that reported among nine species of lizards (*3*). A consistent deviation of body temperature from air temperature cannot be regarded as *prima facie* evidence of regulation, nor can the highest temperatures obtained from a reptile in the field be considered equivalent to a regulated level.

The relation of can temperature to air temperature is indistinguishable from that of heliothermic reptiles. A can exposed to direct sunlight becomes hotter than the surrounding air in the same manner as a reptile. A can, or a lizard, reaches an equilibrium between heat gain and heat loss that results in the maintenance of an elevated body temperature. Although reptiles can regulate their temperature behaviorally, they often reach an equable body temperature and remain at that level passively without need of active regulation (*4*). Therefore, body temperatures randomly collected in the field need not reflect regulation.

If Cowles and Bogert's approach is adequate to demonstrate regulation, at what point in the simplification of procedure was the demonstration of regulation eliminated? Only two of their categories, the maximum voluntary tolerance and minimum voluntary tolerance, contain behavior which alters the heat load upon the animal. The other categories, composed of lethal temperatures and activity and basking ranges, although convenient, are not directly related to active regulation. The elimination of the categories of maximum and minimum voluntary tolerances also eliminated the regulatory elements in Cowles and Bogert's method.

A study of behavioral temperature regulation requires either that the regulatory behavior be witnessed and body temperature immediately measured or that a control be used so that the body temperature of the regulating animal can be compared to the temperature of a model. The cans used in this demonstration would not be adequate as a control because they differ from reptiles in size, shape, reflectivity, and thermal conductance. A simple and useful control might be to tether an animal in the direct sunlight and check its temperature periodically. A deviation of the control temperature from that of an animal collected randomly would be partly attributable to regulation. However, more significant results would come from direct analysis of the regulating behavior.

Further field work must adhere strictly to the methods proposed by Cowles and Bogert. It seems unlikely that the random collection of body temperatures will contribute to further knowledge of temperature regulation among reptiles. The experimental studies of Ruibal (*5*), Lee (*4*), Bartholomew and Tucker (*6*), and Heath (*7*) offer more fruitful approaches.

JAMES EDWARD HEATH
Department of Physiology and Biophysics, University of Illinois, Urbana

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Homocystinuria due to Cystathionine Synthetase Deficiency: The Mode of Inheritance

Abstract. *Deficiency of cystathionine synthetase activity results in the clinical syndrome of homocystinuria. In both parents of a patient with homocystinuria, the hepatic cystathionine synthetase activity was 40 percent of that in unrelated control patients. These findings demonstrate that the metabolic error is inherited and suggest that the parents, although clinically normal, represent the heterozygous state. A second case of homocystinuria also is shown to be associated with cystathionine synthetase deficiency.*

The excretion of homocystine in the urine, homocystinuria, is associated with various clinical features, including mental retardation, dislocation of the ocular lenses, sparse blond hair, genu valgum, convulsive tendencies, failure to thrive, thromboembolic episodes, and fatty change of the liver (*1–5*). In most reported cases, the patients have had, in addition to the high urinary content of homocystine, elevated concentrations of methionine and homocystine in the plasma. We demonstrated previously that the liver of a child with this syndrome lacks activity of cystathionine synthetase and proposed that this enzyme deficiency causes the disorder (*5*). Cystathionine synthetase catalyzes the condensation of homocysteine and serine to cystathionine, this being an intermediate step in the conversion of methionine to cysteine. Absence of the enzyme activity explains the biochemical manifestations of the syndrome.

Recently, Gerritsen and Waisman (*6*) reported that the brain of a homocystinuric patient contained no detectable cystathionine; in children without homocystinuria, cystathionine was present. Although their earlier studies led them to conclude "that the cystathionine synthetase path was not blocked" in this case of homosystinuria (*3*), Gerritsen and Waisman consider their most recent study to be consistent with the absence of hepatic cystathionine synthetase. We think that all their findings in this one case are compatible with a deficiency of this enzyme.

This report concerns the yet unanswered question of whether the error of metabolism is hereditary; if so, what is the mode of inheritance? We also examine the question of whether the enzyme deficiency is invariably associated with mental retardation. This inquiry was prompted by the finding of Barber and Spaeth (*4*) that a paternal cousin of a homocystinuric patient (*4, 5*) excretes homocystine in her urine, but is neither mentally retarded nor otherwise symptomatic.

We assayed hepatic cystathionine synthetase activity in relatives of a homocystinuric patient, C.T. The re-

sults are presented in Table 1, together with the values for the activity of hepatic methionine activating enzyme (*7*) which also is involved in the pathway of methionine metabolism under consideration. The assays for both enzymes were described in our previous report (*5*) and additional details will appear later. In obtaining liver specimens for control values, we were necessarily limited to patients with various diseases. Some of these patients had no detectable hepatic abnormalities; hepatic abnormalities of the others ranged from minor disturbances to extensive metastatic infiltration. The results for all the liver specimens we obtained from living patients are presented in Table 1. These may not reflect precisely the normal range.

Cystathionine synthetase activity varied from 133 to 610 mμmole per milligram of protein, with a mean of 252, ± a standard error of 146. The value for patient 9, 610 mμmole per milligram of protein, differs strikingly from the mean for patients 1 to 8, 207 ± 23. Patient 9 had carcinoma of the pancreas, with extensive metastatic involvement of the liver. Since the mammalian pancreas is rich in cystathionine synthetase activity (*8*), the value for patient 9 may be inordinately high because of the metastatic disease. The results of the methionine activating enzyme assays indicate that the liver specimens were active for that step in methionine metabolism, and that neither the homocystinuric patients nor their relatives had values below the normal range.

The parents of C.T. do not excrete homocystine in the urine, do not have an increased concentration of methionine in the plasma, and do not have any of the clinical stigmata of homocystinuria. Nevertheless, their assay values for hepatic cystathionine synthetase activity were 86 and 88 mμmole per milligram of protein, well below the lowest value of the control group. The mean value for the parents was 42 percent of the mean for patients 1 to 8, and approximately 31 percent of the mean for all nine control subjects. This finding assumes added significance when one notes that both parents had histologically normal livers, whereas several of the control liver specimens showed pathological changes. The maternal grandmother (S.M.) of C.T. appeared normal and had an enzyme activity within the control range.

These several observations support the hypothesis that cystathionine synthetase deficiency is hereditary and is transmitted as an autosomal trait. The heterozygotes, in this case the parents, show no clinical manifestations of the trait despite the reduction in enzyme activity. In contrast, the homozygous state (C.T.) is characterized by greater reduction in enzyme activity and by apparent clinical and biochemical abnormalities. Our studies provide direct evidence for the mode of inheritance suggested by Carson *et al.* (*2*). These authors found that the mother of two siblings with homocystinuria had "a lesser abnormality of methionine metabolism." They concluded that their observations were compatible with the children being homozygous and the mother heterozygous for the gene defect.

Barber and Spaeth (*4*) discovered that M.A.G., an asymptomatic paternal cousin of C.T., excretes homocystine in her urine. Her hepatic cystathionine synthetase activity is 31 mμmole per milligram of protein (Table 1), considerably below the activity of the controls and of C.T.'s parent, but differing significantly from that of C.T. who had no demonstrable activity. Since the value for M.A.G. was low, care was taken to ascertain by means of paper chromatography that the new radioactive compound synthesized during the enzyme assay was cystathionine. This is the second time that we have shown hepatic cystathionine synthetase to be deficient in a patient who excretes homocystine in the urine. Recent studies with animals have shown that dietary and endocrine factors can influence enzymatic activities attributed to cystathionine synthetase (*9*). No relative of C.T. included in this study showed clinical evidence of endocrine disease; all had normal dietary histories and were on a standard hospital diet when biopsied. It is therefore unlikely that such nongenetic factors account for the deviations in enzymatic activity observed in these subjects.

Table 1. Activities of methionine activating enzyme and cystathionine synthetase in extracts of liver specimens obtained from control subjects and from members of a homocystinuric family. Each value for cystathionine synthetase is the average of at least two determinations on a given extract. Individual results are listed in parentheses for members of the homocystinuric family. The values for patients 1, 2, and 9 were reported previously (*5*).

Patient	Age (years), race, and sex*	Clinical features†	Histology of liver biopsy	Enzyme activity (mμmole/mg protein)	
				Methionine activating enzyme	Cystathionine synthetase
		Control patients			
1	55/WM	Psoriasis, alcoholism	Mild fatty infiltration	3.8	204
2	43/WF	Hypoalbuminemia, BSP retention	No pathologic changes	3.5	204
3	32/WM	Fever of unknown etiology	Moderate fatty infiltration and one granuloma‡	6.9	184
4	46/WM	Metastatic carcinoma, primary unknown (?) thyroid	Normal, with extensive metastatic replacement‡	5.1	133
5	42/WF	Rheumatoid arthritis, BSP retention	No pathologic changes	3.3	133
6	64/NM	Rheumatoid arthritis, BSP retention	No pathologic changes	4.6	208
7	60/NF	Adenocarcinoma of the uterus	No pathologic changes‡	3.8	334
8	53/NF	Benign gastric ulcer	Minimal portal fibrosis‡	6.9	219
9	64/WM	Carcinoma pancreas	Metastases‡	Not tested	610
		Homocystinuric patients and family			
C.T.	8/WF	Homocystinuria, with mental retardation	Not examined	7.7	0
D.T.	35/WF	Mother of C.T.; no homocystinuria	No pathological changes	5.0	86 (83,89)
L.T.	38/WM	Father of C.T.; no homocystinuria	No pathological changes	3.9	88 (84,92)
S.M.	56/WF	Mother of L.T.; no homocystinuria	No pathological changes	4.2	257 (265,248)
M.A.G.	24/WF	Paternal cousin of C.T.; homocystinuria but no mental retardation	Normal, with minimal focal fatty change	3.6	31 (30,31)

* N, Negro; W, white; F, female; M, male. † BSP, bromsulphalein. ‡ Liver biopsy performed by laparotomy; all other biopsies were percutaneous.

The study does not define the genetic status of M.A.G. The fact that her enzyme activity falls between those of C.T. and C.T.'s parents could be due to various causes. Although nongenetic factors other than those already mentioned may play a role, the possibilities that multiple alleles or several genetic loci are involved should be considered. Regardless of the mode of inheritance, it is apparent that M.A.G. does not have sufficient cystathionine synthetase activity to prevent her from excreting homocystine in the urine.

The clinically more important question of why M.A.G. is not mentally retarded cannot be answered. Possibly her enzyme level, although low, was sufficient to permit normal mental development; perhaps environmental factors prevented the biochemical defect from causing mental retardation. It has been suggested that an adequate cyst(e)ine content of the diet during the neonatal period may alleviate clinical manifestations of the disease (*2*, *5*). During infancy, C.T. received only cow's milk, a relatively poor source of cystine. For the first 6 weeks of life M.A.G. received human milk, a richer source of cystine.

"Homocystinuria" has been proposed as the name of a disease (*2*). There is, however, a theoretical possibility that excretion of homocystine in the urine may have multiple etiologies; this possibility has already been considered in relation to patients described by Gerritsen and Waisman (*3*). Therefore, we suggest that the term homocystinuria be used to denote excretion of homocystine in the urine, without etiologic connotation. One of the diseases causing this manifestation is the inborn error of metabolism, cystathionine synthetase deficiency.

JAMES D. FINKELSTEIN
National Institute of Arthritis and Metabolic Diseases

S. HARVEY MUDD
National Institute of Mental Health

FILADELFO IRREVERRE
LEONARD LASTER
National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland 20014

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Physical Interaction of a Murine Leukemia Virus with Influenza Virus in vitro

Abstract. *Incubated mixtures of PR8 influenza virus and Rauscher leukemia virus retained the egg infectivity and hemagglutinin of the influenza virus and the ability of the Rauscher virus to induce splenomegaly in mice. Density-gradient centrifugation on potassium citrate gradients revealed a new interviral product with an intermediate density as the principal constituent of such mixtures. Chicken erythrocytes adsorbed the Rauscher virus components as well as the influenza virus components of the interviral product from such mixtures at 4°C, whereas the Rauscher virus alone was little adsorbed. The adsorbed interviral product was eluted from the erythrocyte complex after incubation at 37°C.*

Infection of the same susceptible cell by different viruses can result in pronounced viral interference or synergism. Studies of these effects, however, often involve implicit assumptions that the viruses in the mixture necessarily retain their physical individuality, and that the biological consequences are initiated only on interaction with the cell. In any study of multivirus phenomena it is necessary to check the possibility that the different viruses may have mutually interacted before infecting the cell, and thus provided an effective new set of physical particles having altered biological behavior. This report presents evidence that the PR8 strain of influenza A virus and Rauscher murine leukemia virus readily interact physically in vitro to provide an interviral product having altered density and hemadsorption characteristics.

The Rauscher murine leukemia virus was prepared from the plasma of viremic Balb/c mice that had been previously inoculated with the virus; it was purified and concentrated by a series of differential centrifugations (*1*) so that 1 ml of the final viral concentrate in 0.05*M* sodium citrate solution (*p*H 6.75) was derived from 10 ml of the initial viremic plasma. Influenza virus was similarly isolated and concentrated from the allantoic fluid of embryonated hen eggs that had been inoculated with limiting dilutions of the virus and then incubated for 48 hours. Stocks of the viruses were stored in sealed ampules under nitrogen at −70°C. Before use, the thawed stocks were centrifuged for 2 minutes at 10,000*g* to remove any sediment.

Mixed at approximately 4°C, the viruses were incubated for 30 minutes at room temperature. Assays of the mixture revealed no significant change in the biological titers (egg infectivity and hemagglutinin of the input influenza virus, or the ability of Rauscher virus to cause spleen enlargement in Balb/c mice) compared with those of equivalent dilutions of the separate viruses (Table 1); at first glance no significant interaction of the viral components of the mixture was apparent.

Density-gradient centrifugation of the mixture revealed a somewhat different situation. The Rauscher virus alone, on density-gradient centrifugation for short periods in potassium citrate gradients, formed a single opalescent band (equilibrium density, 1.16 g/cm^3) containing the infectious virus (*2*). Preliminary experiments showed that on similar centrifugation the influenza virus stocks also formed a single opalescent band which coincided with the peaks of the influenza hemagglutinin and infectivity, but which had a distinctly different equilibrium density (1.20 g/cm^3). When, however, an incubated mixture of equal volumes of each virus was centrifuged on a shallow potassium citrate gradient, a new opalescent band appeared which had a density intermediate between those of the separate viruses (Fig. 1). This new intense band was considerably narrower (at 1 mm) than either of the opalescent bands (6 mm wide) formed on separate centrifugation of each virus singly in such identical gradients. Furthermore, the centrifuged mixture showed no significant opalescence in the region normally occupied by the influenza virus