2-1WEAK serums is thought to result from the very small amount of this material present in this particular phenotype.

These various observations all support the contention that the haptoglobin genotypes of the parents of case 2 are Hp^2/Hp^{1WEAK} (father) and Hp^2/Hp^{1S} (mother). Their child with the ring 13 chromosome is believed to have inherited only the father's Hp^{1WEAK} gene. The expected maternal gene was presumably lost during formation of the ring chromosome. Our failure to detect haptoglobin in the serum of case 2 is believed to be consequent to the very limited amount of protein produced by the lone Hp^{1WEAK} gene.

It was previously pointed out that a silent gene in the family of case 1, rather than loss of genetic material during the formation of a ring chromosome, could conceivably have accounted for the anomalous inheritance of haptoglobin in this family (*1*). This was thought unlikely, however, in view of the infrequent occurrence of such a silent gene among Caucasians. A weak gene of the type we postulate for the family of case 2 could not have accounted for the findings on case 1 since the haptoglobin patterns associated with such "weak" genes (Carlberg or 2-1 modified) were not found.

These cases are the only two instances known to us of ring D chromosomes for which haptoglobin studies have been performed and for which autoradiographic analysis has identified the ring D chromosome specifically as chromosome No. 13. Both have been accompanied by anomalous inheritance of haptoglobin type. In each instance the findings may be explained if it is assumed that the locus determining the structure of the haptoglobin alpha-chain is lost during formation of the ring, and hence, that this locus is normally situated on one end or the other of chromosome No. 13.

There have been a limited number of studies of haptoglobin reported for families with other D-chromosome anomalies. De Grouchy *et al.* (*14*) reported the transmission of a D chromosome with a deletion of the short arm from an ahaptoglobinemic father to a son who was heterozygous for haptoglobin. No autoradiographic studies were reported, and, thus, the specific D chromosome involved is not known. If the ahaptoglobinemic state is related to the chromosomal defect in this family, this fact would indicate that a regulatory gene is involved rather than a structural one such as postulated for the two families we have studied.

Hustinx *et al.* (*15*) performed a limited analysis of linkage on families with D/G and D/D translocations and claimed a 14 percent recombination frequency of haptoglobin and the abnormal chromosome. They suggested that the haptoglobin locus is within mappable distance of the centromere in a D chromosome, although in a subsequent report they were unable to show any evidence for linkage after more detailed analysis (*16*). We have performed linkage calculations on the pedigrees of Hustinx *et al.* and on four of our own (two D/G; two D/D); the evidence is inadequate either to confirm or exclude linkage of the haptoglobin locus with the centromere (*17*). None of the families studied by Hustinx *et al.* and only one of ours was analyzed autoradiographically. In the absence of such information the interpretation of this linkage data is questionable.

It is quite probable that the structural locus for the haptoglobin alpha-chain is located on one end of chromosome No. 13; it is not possible to state with certainty whether this locus is on the end of the long arm or the short arm of this chromosome.

GERALD E. BLOOM
PARK S. GERALD
Clinical Genetics Division,
Children's Hospital Medical Center,
and *Department of Pediatrics,*
Harvard Medical School,
Boston, Massachusetts
LEONARD E. REISMAN
University of Louisville,
Louisville, Kentucky

References and Notes

1. P. S. Gerald, S. Warner, J. D. Singer, P. A. Corcoran, I. Umansky, *J. Ped.* **70**, 172 (1967).
2. Haptoglobin is a serum protein that exhibits a genetically determined variation. The genes commonly responsible for this variation are known as Hp^{1F}, Hp^{1S}, and Hp^2. These give rise to the phenotypes 1^F-1^F, 1^F-1^S, 1^S-1^S, 2-2, 2-1^F and 2-1^S. The haptoglobin molecule may be dissociated into subunits during urea-starch gel electrophoresis (a technique known as subtyping). The structural variation associated with this genetic polymorphism has been demonstrated by this means to reside within the alpha-chain subunit.
3. L. E. Reisman, A. Darnell, J. W. Murphy, *Lancet* **1965-II**, 445 (1965).
4. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. M. Hungerford, *Exp. Cell Res.* **20**, 613 (1960).
5. W. Schmid, *Cytogenetics* **2**, 175 (1963).
6. J. German, *J. Cell Biol.* **20**, 37 (1964).
7. O. Smithies, G. E. Connell, G. H. Dixon, *Amer. J. Hum. Genet.* **14**, 14 (1962).
8. P. H. Tarukoski, *Scand. J. Clin. Lab. Invest.* **18**, 80 (1966).
9. J. J. Yunis, E. B. Hook, M. Mayer, *Lancet* **1964-II**, 935 (1964).
10. For the reasons given in this report, the variant gene in this family is believed to be responsible for the production of very little product and hence is referred to as the *1WEAK* gene. Although the haptoglobin pattern in the father of the propositus closely resembles the previously described Carlberg phenotype, it is referred to as a Hp2-1WEAK pattern in this report so as to maintain consistency with the principles of nomenclature used in conjunction with abnormal hemoglobins. In the absence of a specific identification, such as an amino acid substitution, the descriptive terminology 2-1WEAK is preferred. Furthermore, results of subtyping the haptoglobins in this family are slightly different from those on the Carlberg phenotype, so the two may not be identical.
11. F. Galatius-Jensen, *Acta Genet.* **8**, 248 (1958).
12. E. R. Giblett, *Cold Spring Harbor Symp. Quant. Biol.* **24**, 321 (1964).
13. L. Bernini, C. Borri-Voltatorni, M. Siniscalco, *Proc. Int. Congr. Hum. Genet. 3rd*, 5–10 September (1966).
14. J. De Grouchy, Ch. Salmon, D. Salmon, P. Maroteaux, *Ann. Genet.* **9**, 80 (1966).
15. T. W. J. Hustinx, J. B. Bijlsma, L. E. Nijenhuis, *Nature* **207**, 328 (1965).
16. T. W. J. Hustinx, *Cytogenetisch Onderzoek Bij Enige Families* (Brakkenstein, Nijmegen, 1966), pp. 95–99.
17. G. E. Bloom and P. S. Gerald, unpublished data.
18. Supported by NIH grants HE 04706 and HE 5255 and by the John A. Hartford Foundation. Blood grouping studies were done by The Blood Grouping Laboratory, Boston, Massachusetts. We thank Dr. Louis K. Diamond for his helpful suggestions, Dr. Chester Alper for quantitative haptoglobin studies, and William Bucknall for assistance with the linkage calculations.

24 April 1967

Cholinesterase in Plasma: First Reported Absence in the Bantu; Half-life Determination

Abstract. *A Bantu schoolgirl is the first non-Caucasoid found homozygous for the "silent" cholinesterase gene. Investigation of her family revealed two heterozygotes. The propositus possesses no antibody to normal cholinesterase. Transfused cholinesterase had in her a half-life of approximately 10 days.*

The biologic importance of plasma cholinesterase (*Enzyme Nomenclature*: acylcholine acyl-hydrolase, 3.1.1.8) (ACAH) is not well understood, and subjects either completely lacking it or possessing only an atypical form are, under natural conditions, apparently perfectly healthy. However, ACAH is essential for the rapid breakdown of suxamethonium (succinylcholine), a drug frequently used by anesthetists, and, if the normal enzyme is completely absent or replaced by a variant, paralysis and prolonged apnoea, with serious and sometimes fatal consequences, may follow administration of the drug.

The most common variant of the enzyme is recognized by its lower sus-

ceptibility to inhibition by the local anesthetic dibucaine (*1*), while another variant can be identified by its higher resistance to inhibition by fluoride (*2*). The genes responsible for synthesis of the normal or "usual" enzyme (E_1^u) and the two variants to which we have referred (E_1^a and E_1^f) are considered to be allelic. Liddell *et al.* (*3*) drew attention to the existence of a "silent" gene (E_1^s) which, in the homozygous state, resulted in complete absence of ACAH; this is probably an allele of E_1^u and E_1^a (*4*).

Only 11 individuals (in eight families), all Caucasoids, homozygous for the "silent" gene have been described (*3, 5, 6*). We now record the first non-Caucasoid subject lacking ACAH.

A 16-year-old Bantu schoolgirl, B.M., of the Pedi tribe, living in the northern Transvaal, was included in a serogenetic survey. Examined by starch-gel electrophoresis for esterase activity (*7*), her serum showed none of the four isozyme bands normally present. Spectrophotometric assay (*1*) confirmed complete absence of ACAH activity. Samples of serum were obtained from the available relatives for assay and determination of type of ACAH. The mother of the propositus had died about 10 years earlier, aged 40 years, apparently from natural causes and not after an anesthetic.

The results of analyses appear in Table 1. The dibucaine and fluoride numbers indicate the usual form of ACAH (*1, 2*). The sister had a normal level of enzyme; the father, who is an obligatory heterozygote, had a level low in the normal range; while the brother, a presumed heterozygote, had a level about 30 percent of the normal mean for this population.

Table 1. Levels of ACAH, dibucaine and fluoride numbers [by the methods of Kalow and Genest (*1*) and Harris and Whittaker (*2*), respectively], and serum albumin levels of the propositus (B.M.) and her family. The mean enzyme level for 15 Bantu schoolchildren was 0.49 ± 0.15. Activity is expressed as micromoles of benzoylcholine hydrolyzed per milliliter of serum per minute at 25°C.

Subject	ACAH activity	Dibucaine (No.)	Fluoride (No.)	Serum albumin (g %)
Father	0.34	85.3	58.8	4.00
Brother	.14	80.0	66.6	4.20
Sister	.49	85.7	48.9	4.30
B.M.	.00			4.25

All members of the family of B.M. seen by us were healthy on clinical examination; their serum protein levels and electrophoretic patterns were normal. The serum albumin level of the propositus was 4.25 g percent (Table 1); such a normal value in an individual lacking ACAH corroborates the conclusion (*8*) that there is no connection between synthesis by the liver of albumin and of plasma cholinesterase.

It is estimated that the prevalence of subjects homozygous for the "silent" allele is approximately 1:100,000 (*9*). This incidence is considerably lower than the frequency of those homozygous for the dibucaine-resistant gene—1:3000 or 4000 in Britain (*10*). Discovery that this Bantu child is homozygous for the "silent" allele indicates that the gene is not confined to Caucasoids.

Lehmann and Liddell (*11*) found that the serum of a patient completely lacking ACAH had an inhibitory effect on the ACAH activity of normal serum, but Hodgkin *et al.* (*6*) could not confirm this finding in their two patients. Our identical experiment with the serum of B.M. failed to demonstrate inhibition of activity. This fact may indicate that different genetic defects occur in the two groups; on the other hand, the difference may reflect the production of antibodies against ACAH by the patient of Lehmann and Liddell (*11*), who had been transfused earlier and may have been sensitized to the ACAH contained in the plasma. However, our patient's blood showed no inhibition of normal serum ACAH activity when the experiment was repeated 7 months after a transfusion.

We performed an experiment (*12*) that gives some indication of the rate of turnover of ACAH in the body. Blood (about 200 ml) was taken from the propositus before she received, over a 20-minute period, 460 ml of freshly collected plasma from two donors possessing the usual type of ACAH in normal amounts (0.42 and 0.57 units, respectively; see Table 1). Samples of blood were collected within 2 minutes of completion of the infusion (zero time) and thereafter at varying intervals.

The result (Fig. 1) shows that after the initial rapid drop in enzyme level, due to distribution in the tissues, there was a more gradual decrease in activity and a half-life of about 10 days. Results have been similar for the regeneration rate of plasma cholinesterase after the administration of diisopropyl fluorophosphate (*13*).

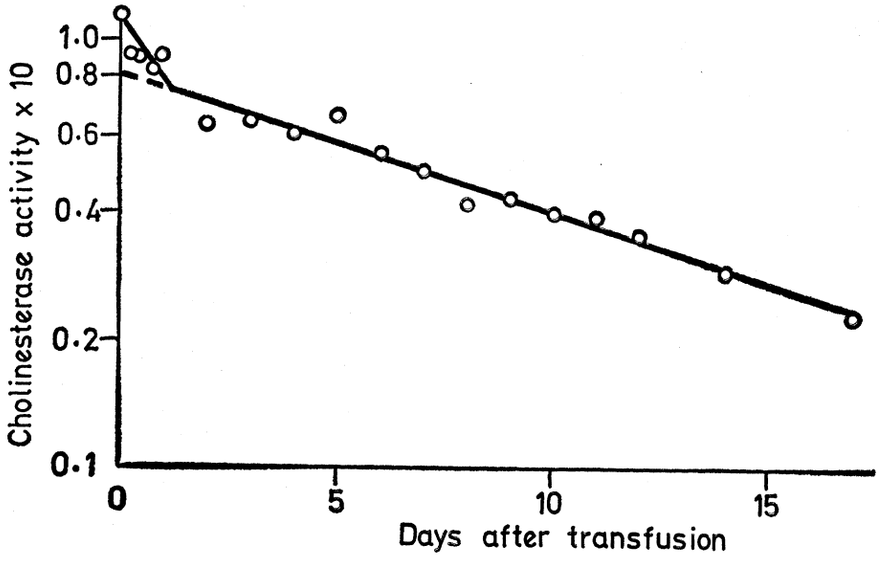

Fig. 1. Change in ACAH level in serum of a subject, lacking the enzyme, after transfusion of normal plasma (semilogarithmic plot).

TREFOR JENKINS
DORIS BALINSKY
South African Institute for Medical Research, Johannesburg, Transvaal
D. W. PATIENT
Jane Furse Memorial Hospital, Sekhuniland, Transvaal

References and Notes

1. W. Kalow and K. Genest, *Can. J. Biochem.* **35**, 339 (1957).
2. H. Harris and M. Whittaker, *Nature* **191**, 496 (1961).
3. J. Liddell, H. Lehmann, E. Silk, *ibid.* **193**, 561 (1962).
4. N. Simpson and W. Kalow, *Amer. J. Human Genet.* **16**, 180 (1964).
5. H. W. Goedde, W. Fuss, H. Ritter, H. Baitsch, *Humangenetik* **1**, 311 (1965); T. G. Doenicke, G. Kreutzberg, I. Remes, W. Spiess, K. Steinbereithner, *Acta Anaesthesiol.*

Scand. **7**, 59 (1963); A. Szeinberg, S. Pipano, E. Ostfeld, L. Eviatas, *J. Med. Genet.* **3**, 190 (1963); A. A. Dietz, T. Lubrano, H. M. Rubinstein, *Acta Genet. Basel* **15**, 208 (1965).
6. W. E. Hodgkin, E. R. Giblett, H. Levine, W. Baker, A. G. Motulsky, *J. Clin. Invest.* **44**, 486 (1965).
7. J. Stern and W. H. P. Lewis, *J. Mental Deficiency Res.* **6**, 13 (1962).
8. A. A. Dietz, H. M. Rubinstein, T. Lubrano, *Clin. Chem.* **12**, 25 (1966).
9. A. G. Motulsky, in *Progress in Medical Genetics*, A. G. Steinberg and A. G. Bearn, Eds. (Grune and Stratton, New York, 1964), vol. 3, p. 49.
10. C. Kattamis, E. Zannos-Mariolea, A. P. Franco, H. Liddell, H. Lehmann, D. Davies, *Nature* **196**, 599 (1962).
11. H. Lehmann and J. Liddell, in *Progress in Medical Genetics* (9), vol. 3, p. 75.
12. At the suggestion of K. B. Cooke, Westminster Hospital, London.
13. D. Grob, J. L. Lilienthal, Jr., A. M. Harvey, B. F. Jones, *Bull. Johns Hopkins Hosp.* **81**, 217 (1947); H. W. Neitlich, *J. Clin. Invest.* **45**, 380 (1966).
14. We thank A. Zoutendyk, I. Bersohn, and T. Ipp for help and B.M. for cooperation. One of us (T.J.) received a grant from the South African Council for Scientific and Industrial Research.

3 April 1967

Adenyl Cyclase Activity in Rat Pineal Gland: Effects of Chronic Denervation and Norepinephrine

Abstract. *Adenyl cyclase activity in the pineal gland of rats was determined by measuring the rate of formation of radioactive cyclic 3′,5′-adenosine monophosphate from ^{14}C-labeled adenosine triphosphate. Norepinephrine added in vitro to pineal homogenates enhanced this activity, while denervation of the pineal gland by superior cervical ganglionectomy did not significantly reduce it. The enzyme in these denervated glands was more responsive to the stimulatory effects of norepinephrine.*

Over the past several years there has been an accumulation of evidence which indicates that cyclic 3′,5′-adenosine monophosphate (cyclic 3′,5′-AMP) is a mediator of certain hormone-induced responses (*1*). The enzymes that catalyze the formation (adenyl cyclase) (*2*) and the hydrolysis (cyclic nucleotide phosphodiesterase) (*3*) of cyclic 3′,5′-AMP are widely distributed in mammalian and nonmammalian tissues. The evidence that catecholamines stimulate adenyl cyclase activity (*4*) suggests that in some tissues adenyl cyclase functions as a receptor for these amines. Since chronically denervated tissues are at least as responsive to catecholamines as innervated tissues are (see *5*), adenyl cyclase must be situated distally to nerve endings if it is to qualify as such a receptor. Loss of adenyl cyclase activity after denervation would indicate that the enzyme is not involved in catecholamine-induced responses at postjunctional sites. We have, therefore, investigated this activity in innervated and chronically denervated structures to determine the location of the enzyme and to examine its responsiveness to catecholamines.

The pineal gland is ideally suited for studying the changes in adenyl cyclase activity that might occur with chronic denervation. This organ is innervated exclusively by sympathetic fibers originating bilaterally from the superior cervical ganglia (*6*). Thus, superior cervical ganglionectomy causes degeneration of the pineal nerves (*6, 7*) and depletion of the catecholamines stored in its nerve terminals (*8, 9*), as shown by microscopic observation of the gland and by direct chemical analysis of norepinephrine.

Adenyl cyclase activity was determined by measuring the rate of accumulation of radioactive cyclic 3′,5′-AMP formed from its labeled precursor, ^{14}C-adenosine triphosphate (ATP). Cyclic 3′,5′-AMP is separated from ATP and other metabolites of ATP in two separate stages. The compounds are adsorbed onto ion-exchange columns [Dowex 50W-X4(H^+)] and eluted with 1-ml portions of water. This procedure eliminates approximately 99.9 percent of ATP from the cyclic 3′,5′-AMP fraction. Final purification of this fraction is achieved by precipitating trace contaminants by adding equal volumes of barium hydroxide (7.2 percent) and zinc sulfate (8 percent). Approximately 75 percent of the cyclic 3′,5′-AMP is recovered in a final volume of 3 ml, and the fraction is pure enough to enable detection of one part of the cyclic nucleotide in more than 50,000 parts of ATP. Moreover, rate of accumulation of the cyclic nucleotide in rat brain homogenates is linear with respect to both time and concentration of enzyme, if proper precautions are taken to maintain adequate substrate concentrations and to prevent the enzymatic hydrolysis of cyclic 3′,5′-AMP (*10*). An account of the present experiment has recently been reported (*11*).

In the present experiments, adenyl cyclase activity of pineal glands of male Sprague-Dawley rats (200 to 240 g) was determined as follows. Within 30 seconds after the rats were decapitated, each organ was removed, homogenized in tris-HCl buffer ($5 \times 10^{-2}M$, *p*H 7.4), and incubated individually in 100 μl of an assay mixture described in Table 1 and Fig. 1. Tubes were incubated at 30°C in a Dubnoff metabolic shaker. The reaction was stopped by immersing the tubes in boiling water for 5 minutes; carrier cyclic 3′,5′-AMP was added, and, after purification, the specific activity of the cyclic nucleotide in each sample was determined by measuring its radioactivity with a liquid scintillation spectrometer and its optical density, at 260 mμ, with a spectrophotometer.

A study of the time course of the accumulation of cyclic 3′,5′-AMP-^{14}C in pineal homogenates incubated with ATP-^{14}C indicated that cyclic 3′,5′-AMP was formed rapidly and destroyed rapidly and that the rate of formation was enhanced by norepinephrine. For

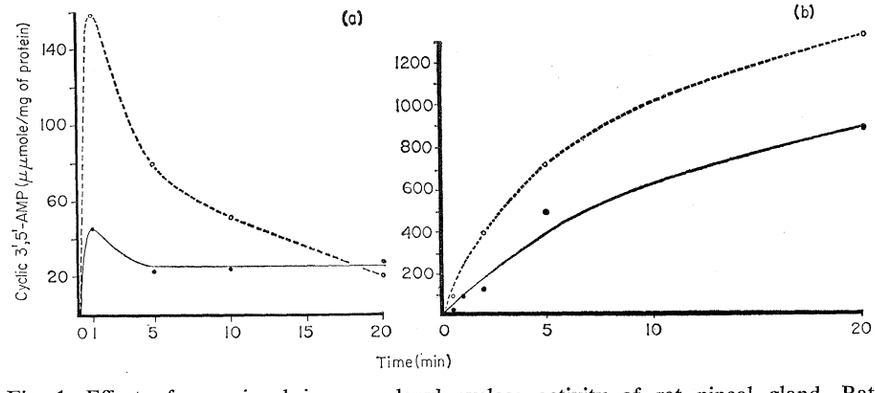

Fig. 1. Effect of norepinephrine on adenyl cyclase activity of rat pineal gland. Rat pineal homogenates were assayed for adenyl cyclase activity by measuring radioactive cyclic 3′,5′-AMP formed from ^{14}C-ATP. (a) Each incubation tube contained: 1 pineal, 1 μc of ATP-8-^{14}C ($2 \times 10^{-3}M$), and Mg^{++} ($3 \times 10^{-3}M$) in 100 μl of tris-HCl buffer ($5 \times 10^{-2}M$, *p*H 7.4). (b) Same as (a) except for the addition of theophylline ($10^{-2}M$) to prevent the enzymic hydrolysis of cyclic 3′,5′-AMP. Solid lines, without norepinephrine; dashed lines, with *l*-norepinephrine bitartrate ($1 \times 10^{-4}M$). Each point represents one determination.