Table 2. Data on correct and incorrect answers in history of science test: eight sets of names to be arranged in chronological order.

Names	Number		
	Correct	Incorrect	Blank
1. Faraday, Hertz, Maxwell	10	30	12
2. Copernicus, Galileo, Newton	23	23	6
3. Berzelius, Kekule, Pasteur	12	26	14
4. Weierstrass, Cantor, Dedekind	3	24	25
5. Mendeleef, Dalton, Lavoisier	12	30	10
6. Darwin, Lamarck, Linnaeus	13	30	9
7. Leibniz, Descartes, Gauss	16	20	16
8. Leeuwenhoek, Schwann, Koch	19	21	12

man" or "a physical reality independent of the observer"; the terms used were *trees, mountains, stars, genes, gravity, photons, molecules,* and *species.* There was virtually no change in response to most of the items in this question or in the number of items designated as "concepts" by the fellows (pretest median = posttest median = 2). Two interesting shifts in opinion took place: in the posttest, seven more fellows saw *genes* as concepts than in the pretest, and seven more saw *photons* as physical realities. Was a move toward "naive realism" in the way they looked at the photon coupled with a move toward "logical positivism" in the way they looked at the gene?

In a related question, the fellows definitely shifted away from the view that mathematical axioms are "self-evident truths" and toward the view that such axioms are "arbitrary conventions."

These results suggest that one can fairly easily inculcate specific changes in thought that have little general effect on the individual's approach to science as a whole. Philosophical issues were not discussed enough in the program to permit much clarification; the changes that did occur resulted from the specific ways in which different faculty members handled certain topics in their classes. One of the fellows, in fact, commented on "knowing the right answer" from attending the seminar in mathematics. The larger issue—of developing some mature appreciation of the history and philosophy of science—has yet to be dealt with; meanwhile, it is perhaps encouraging that some changes in this domain of thought can be made.

Evidence that the fellows had not learned to teach students much about the way in which scientific progress is achieved was given by their sample teaching performances. Each of a representative group of fellows gave a half-hour talk on a topic of his own choice designed for a high school classroom. In these short talks the fellows were almost entirely preoccupied with presenting the known facts and principles of science and mathematics. Very seldom was any effort made to convey a sense of the *way* in which scientific thought unfolds —the thinking and research lying behind the material the teacher was presenting. The history leading up to a scientific discovery and the consequences of such a discovery were never discussed. Within these limitations many of the talks were fluent, interesting, and well organized. There was no change in performance from the beginning of the year to the end of it, except that on occasion the later talks drew on specific material learned in the Academic Year Institute.

It may be that the institute's preoccupation with the goal of teaching specific science material prevented it from dealing adequately with other goals envisioned for the program—envisioned, at least, by the faculty concerned. The working assumption of the institute in its initial year was that if subject matter is adequately taught, other things will take care of themselves. The main conclusion of the evaluation group is that such an assumption is at best questionable.

Science plays an increasingly large part in every individual's life; if it reaches him only through its technological fruits, man will be increasingly divorced from nature, and scientific progress will, paradoxically, impoverish his intellectual and cultural life; but if the study of science gives man a deeper appreciation of nature and increased ability to enjoy the pleasures of rational thought, it will ennoble and enrich him. To accomplish these ends, science teachers must do more than study what scientists know; they must understand how scientists think.

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Notes

This study was supported by the Academic Year Institute at the University of Colorado, with funds from the National Science Foundation. For a more detailed report of the methods and results of the evaluation, see H. E. Gruber, K. P. Brady, and J. R. Means, "Toward the Improvement of Science Teaching," *University of Colorado Behavior Research Lab. Rep. No. 9.* The findings reported here led the evaluation group, in cooperation with the Academic Year Institute faculty, to develop and test experimentally a method of overcoming the difficulties described.

For related discussion, see P. F. Brandwein, F. G. Watson, P. E. Blackwood, *Teaching High School Science: A Book of Methods* (Harcourt, Brace, New York, 1958); J. B. Conant, *On Understanding Science* (Yale Univ. Press, New Haven, 1947); J. R. Oppenheimer, *Physics in the Contemporary World* (Anthoesen, Portland, Me., 1947).

20 April 1960

Biosynthesis of Chick Hemoglobins

Abstract. In vivo studies of the biosynthesis of chick hemoglobins 1 and 2 showed an over-all higher incorporation of glycine-2-C^{14}, valine-4-C^{14}, valine-1-C^{14}, leucine-G-C^{14}, and histidine-(2-ring)-C^{14} in hemoglobin 2; in vitro studies made with intact nucleated chick erythrocytes showed the higher incorporation of glycine-2-C^{14}, valine-1-C^{14}, and histidine-(2-ring)-C^{14} in hemoglobin 1. Hybridization of chick hemoglobins produced an electrophoretically distinguishable new component.

Paper electrophoretic investigation on the cell-free hemolyzates obtained from the erythrocytes of birds like chick, guinea fowl, and duck revealed the presence of two hemoglobins (*1*). It has further been observed that in chick one of these hemoglobins (Hb 1) decreases while the other (Hb 2) increases during development (*2*). Both of these hemoglobin components were found to be of the alkali-resistant type (*3*). In view of these findings, it was of interest to investigate the biosynthesis of individual hemoglobin components of chick. This report presents the results of the investigation carried out with adult chicks injected with radioactive amino acids, and the in vitro incorporation of C^{14}-labelled amino acids in the hemoglobin components by the nucleated chick erythrocytes.

For in vivo studies, 6-month-old male white leghorn chicks were intravenously injected with 25 μc of amino acid-C^{14}. Thereafter, 4 ml of heparinized blood was collected from each chick on alternate days for 2 weeks and then every 4th day until the 28th day after the injection. Blood samples were washed four times with ten times their volume of isotonic saline at 2°C in a refrigerated centrifuge. Clear hemoglobin solutions were obtained by hemolysis with water and toluene and by centrifugation as described earlier (*1*).

For in vitro studies, chicks were made mildly anemic with injections of acetylphenylhydrazine. Acetylphenylhydrazine (25 mg) was injected subcutaneously three times, once every 4th day, followed by an initial withdrawal of 15 ml of blood. After this, approximately 10 ml of blood was withdrawn and 20 mg of acetylphenylhydrazine was injected every week. Heparinized blood samples were obtained from these birds 48 hours after the injection of acetylphenylhydrazine. During the washing procedure, care was taken not to remove the light colored upper layer which contained reticulocytes. In the in vitro experiments, to one volume of erythrocytes equal volumes of normal chicken plasma and a synthetic media containing an amino acid mixture as described by Borsook (*4*) (glucose, 1.0 mg/ml; Na-penicillin G and strepto-

mycin sulfate, each, 0.1 mg/ml; $FeSO_4$, $(NH_4)_2SO_4,6H_2O$, 5 μg/ml; $MgCl_2$, 2.85 mg/ml; α-ketoglutaric acid, 10 mg/ml; pyridoxal-5-phosphate, 17 μg/ml; and 5 to 10 μc of glycine-2-C^{14}, leucine-G-C^{14}, valine-1-C^{14}, valine-4-C^{14} and histidine-(2-ring)-C^{14}) were added. Incubation was carried out at 37°C for 4 hours, in air. After incubation, the mixtures containing chick erythrocytes were washed six times with ten times their volume of isotonic saline, and hemoglobins were extracted as described previously.

Paper electrophoresis was conducted in barbiturate buffer (*p*H, 8.6; μ, 0.05) for 16 to 18 hours with 220 volts at 4°C in a horizontal paper electrophoresis apparatus. After the paper electrophoretic run was over the peak areas were cut out and eluted with water in a water-saturated, all-glass, chromatographic chamber. Since the concentration of hemoglobin 1 is always higher than that of hemoglobin 2, the eluted solutions containing hemoglobin 1 were diluted approximately to the concentration of hemoglobin 2 fractions in order to minimize errors in measurement of hemoglobin content and of specific radioactivity. The hemoglobin content of the fractions was determined as carbomonoxyhemoglobin at 540 mμ in a Beckman DU spectrophotometer, and radioactivity was determined with a windowless gas-flow counter. The results were calculated as the specific activity, counts per minute per milligram of hemoglobin, divided by the number of micromoles of a particular amino acid present per milligram of the hemoglobin component. The amino acid content used in calculation was based on the results reported by Helm and Huisman (*5*).

Table 1 presents the ratio of the specific activity of the chick hemoglobins 1 and 2 corrected for the differential amount of amino acids present in each component during the period of in vivo studies. It may be observed that throughout the period of investigation, except for the 2nd- and 4th-day results, hemoglobin 2 shows an over-all higher specific activity than hemoglobin 1. The results are in agreement with the earlier observation that during development chick hemoglobin 2 increases from 29 percent to 39 percent, while hemoglobin 1 decreases from 73 percent to 61 percent (*2*). However, the observed higher amino acid–C^{14} incorporation in component 1 in the first days of analyses could not be explained as a result of preferential production of one hemoglobin component by the living system during development. It may, however, be pointed out that paper electrophoretic analyses undertaken on a large number of chicks representing the different age groups starting from the embryonic chicks to adult ones always revealed the presence of both the hemoglobins. Similar in vivo experiments with leucine-G-C^{14} were performed on guinea fowl, and the results obtained resemble those reported with chicks.

Table 1. In vivo biosynthesis of chick hemoglobins 1 and 2. Values are ratios of the rate of amino acid–C^{14} incorporation in hemoglobin 2 to the rate in hemoglobin 1. Rates were calculated from specific activity per mg of hemoglobin corrected to 1.0 micromole of the particular amino acid present in each hemoglobin component.

Days after injection	Glycine -2-C^{14}	Leucine -G-C^{14}	Histidine-(2-ring)-C^{14}	Valine -1-C^{14}	Valine -4-C^{14}
2	0.86	*	*	1.35	1.00
4	1.01	0.75	1.00	1.37	1.00
6	1.38	1.53	2.25	1.74	2.59
8	1.26	2.08	1.76	0.95	1.06
10	1.77	1.50	1.82	1.08	1.11
12	1.48	1.47	1.74	1.07	1.10
14	1.85	1.30	1.20	0.97	1.18
16	1.44	1.04	2.16	0.99	1.17
20	1.53	1.13	1.13	0.82	1.19
24	1.36	1.06	1.57	0.91	1.10
28	1.99	1.33	1.07	0.73	1.24
32	1.39				
36	1.58				
Over-all ratio	1.45	1.31	1.62	1.08	1.23

* Specific activities were too low to be recorded.

Table 2 shows that, unlike the in vivo system, the chick erythrocytes incorporate amino acid–C^{14} more in component 1. The rate of incorporation was found to be higher in the anemic birds but the ratio of incorporation in hemoglobin 1 to that in hemoglobin 2 does not show any remarkable change from the ratio obtained with the normal birds. Thus it appears that the erythrocytic production of hemoglobins brings about higher incorporation of radioactive amino acid in hemoglobin 1 during the first days of in vivo studies. It seems that while the nucleated chick erythrocytes synthesize greater amounts of hemoglobin 1, the hemopoietic system as a whole reverses the ratio of the erythrocytic production of hemoglobins 1 and 2. According to the template theory of protein synthesis polypeptide chains are synthesized in microsomal nucleoprotein templates containing ribonucleic acid, which in turn are organized by chromosomal deoxyribonucleoproteins. The present investigation reveals that both the genes which control the molecular conformations of hemoglobins 1 and 2 are present in the chick erythrocytes and so also are the rate-determining factors controlling the production of these hemoglobins. Differences in the composition or in the state of internal milieu probably determine to a large extent the relative rates of hemoglobin synthesis.

Hybridization of chick hemoglobins 1 and 2 was carried out with a method similar to that outlined by Itano and Singer (*6*). A new component with a mobility intermediate between the positions of hemoglobins 1 and 2 appears (Fig. 1). Similar results were obtained with pigeon and duck hemoglobins. This observation provides evidence of the dissociation of chick hemoglobins 1 and 2 into two unlike subunits in each case. Oxygen equilibria studies of chick hemoglobins dissolved in urea (4.0*M*) and sodium chloride (4.0*M*) also indicated the dissociation into half

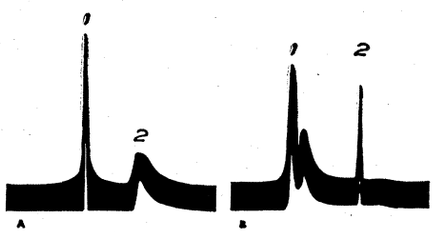

Fig. 1. Ascending boundary patterns of free electrophoresis of chick carbonmonoxyhemoglobins 1 and 2 in Na-phosphate buffer, *p*H 6.8; μ 0.02. (*A*) control; (*B*) test; hemoglobins 1 and 2 were dissociated in Na-acetate buffer, *p*H 4.7; μ, 0.2 for 5 hours at 3°C and returned to Na-phosphate buffer, *p*H 6.8; μ, 0.02.

Table 2. In vitro biosynthesis of chick hemoglobins 1 and 2. Values represent rates of incorporation. A 3 ml volume of packed erythrocytes was suspended in 6 ml of medium containing equal amounts of normal chicken plasma and synthetic medium. Incubation was carried out in 50 ml erlenmeyer flasks in Warburg apparatus which was modified to produce a rotary shaking of 100 cycles per minute.

Normal chick			Anemic chick		
Hb 1	Hb 2	Hb 1/ Hb 2	Hb 1	Hb 2	Hb 1/ Hb 2
Valine-1-C^{14}					
16.6	8.0	2.0	47.8	21.6	2.2
Histidine-(2-ring)-C^{14}					
27.0	8.0	3.3	45.0	20.0	2.5
Glycine-2-C^{14}					
19.7	13.2	1.5	100.4	35.4	2.8
13.1	4.3	3.0	245.7	168.0	1.4
49.1	42.1	1.2*	192.6	130.3	1.5*
94.5	64.3	1.5†			

* Experiments were conducted with 2-week-old chicks. † Erythrocytes were incubated in plasma alone.

molecules (*7*). Presumably there are units in these molecules which may be represented by α_1 α_1 and β_1 β_1 in chick hemoglobin 1, and α_2 α_2 and β_2 β_2 in chick hemoglobin 2. Since only three components are detected electrophoretically, it seems that either $\alpha_1\alpha_1$- and $\alpha_2\alpha_2$-, or $\beta_1\beta_1$- and $\beta_2\beta_2$-units of polypeptide chains are identical. One may wonder, however, whether these dissociations and recombinations are possible in the animal system. Results obtained on the in vitro and in vivo biosyntheses of chick hemoglobins suggest the possibility of the transfer of one of the subunits from one of the hemoglobins to the other. On the other hand, the rate of production of these three subunits may be genetically so controlled that they lead to results like those demonstrated here. However, mutations in any one of these genes would give rise to numerous hemoglobins in the avian species which have been experimentally observed earlier (*1, 8, 9*).

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Simultaneous Study of Behavior and Brain Waves

Abstract. A technique for the simultaneous audiovisual recording of behavior and brain waves is described. The absence of muscle movement artifact, despite unlimited activity of the patient, suggests that telemetering may be adaptable for routine electroencephalography.

This preliminary report describes our development of an audiovisual technique for the simultaneous study of a patient's behavior and electroencephalogram (EEG).

In the Children's Service of the Langley Porter Neuropsychiatric Institute, where research is primarily concerned with defining the etiologic role of experiential factors in childhood schizophrenia (*1*), the possible contribution of any organic factor in all children seen is studied with all the facilities and skills available, since the differentiation between schizophrenic children and emotionally disturbed children with mental deficiency or organic brain disease may be initially difficult. We occasionally find EEG dysrhythmias in children of varied diagnoses without any historical or clinical evidence of a convulsive disorder. For such cases we were particularly interested in devising a technique for the more precise study of the correlation, if any, between behavior and dysrhythmias.

In 1951 Berlin and Yeager (*2*) noted that the level of emotional tension in epileptic children may be correlated with the frequency and severity of seizures as well as the degree of EEG dysrhythmia and, in 1956, Yeager and Guerrant (*3*) reported that a patient's performance, as measured by finger tapping, was altered sometimes during a paroxysmal burst on the electroencephalogram without any other apparent clinical evidence suggesting seizure activity.

In 1958 (*4*), a transistorized telemeter about the size of a "king-size" package of cigarettes was developed by the Research and Development Laboratory of the University of California Medical Center, San Francisco. The telemeter shown in operation on a child in Fig. 1 uses a circuit, previously described in detail (*4*), which consists of a four-stage transistor amplifier, a reactance modulator, and a 30-Mcy/sec oscillator which produces signals frequency-modulated by the patient's scalp voltages. The brain waves may be telemetered to an antenna within a radius of 40 feet and then coupled by a frequency-modulated receiver to a conventional electroencephalograph. Either needle or disk scalp electrodes may be used. Thus a trace can be made while the child, unencumbered by leads, is able to move about freely.

Preliminary trials with the telemeter produced traces *with a surprising absence of muscle movement artifact* (Fig. 1 includes such a trace). We then considered how a 16 mm motion picture camera might be utilized to photograph the child and the telemetered electroencephalogram on the same film.

With a motor-driven 16 mm camera with masks behind the lens which allowed exposure of either two-thirds or one-third of each film frame, we photographed the child, notching the film at a landmark in the camera before starting the motor. The instant the camera motor was started the EEG trace was marked. The upper third of the film was masked for this first exposure.

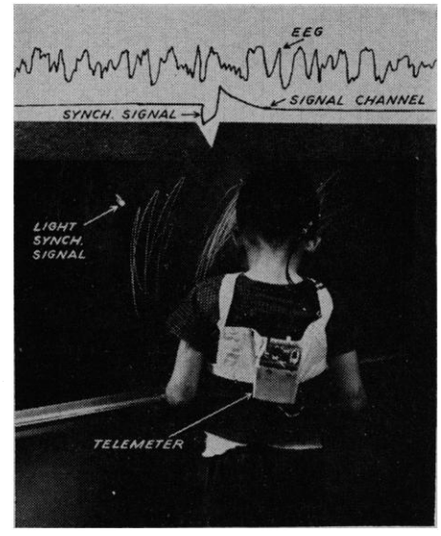

Fig. 1. Effect achieved in a frame of the motion picture film. The composite photograph was prepared because of technical difficulties involved in enlarging directly from the film.

After 100 feet of film were exposed without interruption, the film was rewound in a darkroom, replaced in the camera, and the notch in the film was aligned with the same landmark as before. The EEG trace was also rewound to the mark made when the camera motor was started, and the lens mask was changed to expose the remaining third of each film frame. The camera and EEG motors were then started simultaneously.

Although our first film was satisfactory photographically, we had no demonstrable proof that synchronization of the child's behavior and the electroencephalogram was either initially correct or was maintained.

We then decided to use a sound camera and a system to check synchronization.

Using this system, we modulated the electroenecephalogram into an audible frequency directly recorded on the sound track at the first filming. We also devised a signal system in which pressing a button flashed a light on the wall of the playroom, produced an audible click in the modulated electroencephalogram, and marked the EEG trace on a second channel. This signal was triggered by one of us each time a paroxysmal burst occurred on the electroencephalogram (a frequent occurrence with the patient studied, who manifested a hypsarhythmic record).

Our technique of marking the film and the electroencephalogram, masking the film and rewinding it and the EEG trace after the initial exposure, and