ble than among the controls. The data indicate that in the presence of a schizophrenic psychosis increased and highly variable adrenal activity is a common occurrence, and is apparently unrelated to psychomotor activity.

Summary. These investigations imply that emotional disorders are accompanied by changes in protein metabolism and adrenal activity. The protein metabolic disturbances are generally in the direction of increased catabolism, being well correlated with emotional response when the disorder is essentially an anxiety reaction to acute stress, less well correlated in schizophrenic psychosis, but sometimes still pronounced and, finally, lacking in correlation in the presence of conversion hysteria. Furthermore, a comparison of schizophrenic psychotics with normal controls reveals that the former exhibit a generally higher and more variable state of adrenal activity. It is hoped that these ostensibly unrelated sets of data may eventually become integrated with a coherent concept of individual reactions to stress.

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Rapid CO_2 Determination with the Beckman O_2 Analyzer.* (19088)

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The current daily hospital routine, which includes Tissot basal metabolism, maximum breathing capacity and exercise tests, requires from 40 to 60 expired air analyses in duplicate. The classical Haldane technic has been the method of choice for many years. Since 1947, efficiency has been increased through the innovation of O_2 analysis, based upon the paramagnetic qualities of O_2 so ingeniously devised by Pauling(1). Consequently, CO_2 has been determined as usual by Haldane, with only a complete duplicate analysis as a check on 1 out of every 10 determinations on the Beckman O_2 analyzer. To further minimize Haldane analysis, the search for a cheap, rapid, CO_2 method accurate enough for this purpose has been our objective in recent years. Infra-red spectroscopy(2,3) and the mass spectrograph(4) are especially suited to accurate, quick, CO_2 determination in continuous analysis but are elaborate and expensive. Although a variation of the simpler and cheaper venturi meter method(5) has been tried and a conductivity apparatus(6,7), which we have built into a constant temperature chamber, is now in use, the principle of

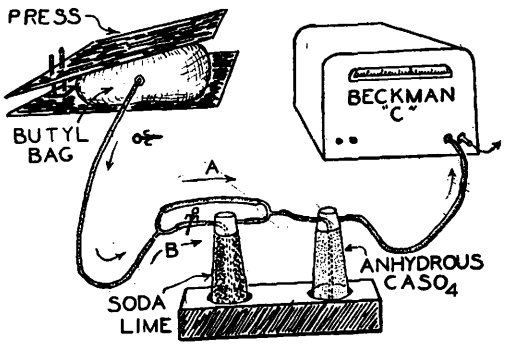

FIG. 1.

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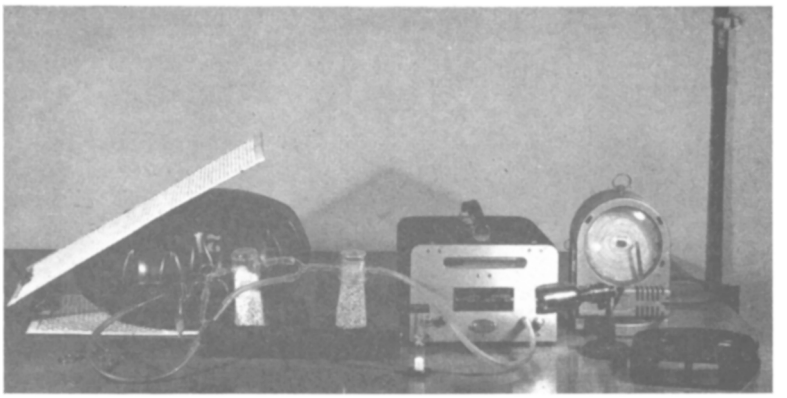

FIG. 2.

the CO_2 method to be presented in this report was disclosed rather fortuitously as a consequence of our early experience in magnetic O_2 analysis. In the conversion from the classical Haldane Hg sampling tubes to bags, suitable for paramagnetic gas analysis, a gradual apparent increase in the O_2 values, resulting from CO_2 loss through the rubber, was observed. Transfer to synthetic butyl bags, which are practically impervious to CO_2 (approximate loss of 0.01-0.03% CO_2/24 hr) allowed O_2 values consistent with Haldane results. This experience suggested the idea that CO_2 calculation might be possible if the apparent O_2 increase on complete CO_2 removal were significant. Since this proved to be true, the feasibility and accuracy of this method has been tested.

The procedure is shown diagrammatically in Fig. 1. In step A the gas is pressed from its butyl bag, at an optimum flow rate of 200 cc per minute for 2½ minutes through anhydrous $CaSO_4$ to the Beckman analyzer, (C model) resulting in the reading of the O_2 tension in mm Hg partial pressure, which is readily converted to O_2 percentage. Without delay the O_2 percentage after CO_2 removal (step B) is similarly determined by diverting the gas through soda lime and anhydrous $CaSO_4$ into the analysis chamber. The procedure was facilitated by a continuous barometric pressure record (Baroscribe), a 2½-minute timer, a small lamp, and a magnifocuser (a binocular loupe, which magnifies 3x) to increase accuracy of scale reading, as depicted in Fig. 2.

The efficiency of ascarite, KOH solution (17%) and soda lime in absorption bulbs of variable size and shape was tested. Ascarite, though highly effective as a quantitative CO_2 absorbent, tended to solidify hindering the flow rate. KOH solution was also satisfactory, but to avoid the damage which might result if the alkaline reagent entered the gas chamber, the equally efficient and cheap soda lime (mesh No. 4-8, moisture content 4-19%) was preferable. Anhydrous $CaSO_4$ (indicating drierite, mesh No. 8) proved to be an efficient agent for moisture removal. The drierite (100 g) was changed when two-thirds of it had turned pink, usually after 100-150 determinations. The soda lime (100 g) remained effective for approximately 450-500 CO_2 analyses.

CO_2 calculation was made by the following simple relationship: Let $A = O_2\%$; $B = O_2\%$ after CO_2 removal; $B = 100\ A/100 - CO_2\%$; $CO_2\% = 100\ (B\text{-}A)/B$.

Data was accumulated in duplicate on 300 expired air samples, using Haldanes, the Beckman E-2 model and the Beckman C model O_2 analyzers. Considering that a small CO_2 percentage is determined by the difference between two relatively large O_2 percentages, the results have proved to be quite consistent. Results on 10 consecutive samples are shown in Table I. On the basis of averages, the average deviation from the Haldane is practically identical for both models (0.065 for C and 0.069 for E-2) but the variation between duplicate CO_2 values is definitely greater in the E-2 data. It is true that the accuracy of the CO_2 value depends

TABLE I. Consecutive CO_2 % Determinations in Duplicate.

Sample No.	Haldane	Avg	Beckman C model	Avg	Beckman E-2 model*	Avg
1	1.93	1.91	1.99	1.99	1.89	1.81
	1.89		1.99		1.73	
2	2.43	2.41	2.43	2.46	2.54	2.46
	2.39		2.49		2.38	
3	3.19	3.18	3.19	3.16	3.24	3.12
	3.17		3.13		3.07	
4	3.46	3.49	3.43	3.43	3.49	3.52
	3.52		3.43		3.54	
5	2.80	2.78	2.83	2.86	2.83	2.80
	2.76		2.89		2.77	
6	3.71	3.70	3.74	3.69	3.85	3.88
	3.69		3.64		3.90	
7	3.58	3.57	3.50	3.47	3.67	3.65
	3.55		3.44		3.62	
8	3.85	3.84	3.67	3.69	3.81	3.75
	3.83		3.70		3.69	
9	3.17	3.18	3.09	3.11	3.08	3.17
	3.19		3.12		3.26	
10	3.26	3.24	3.18	3.21	3.18	3.24
	3.22		3.24		3.29	

* Experience with the E-2 model has been less satisfactory, as indicated by the wide variation between duplicates.

upon the care with which the O_2 readings are made. Therefore, it follows that the sensitivity as well as the percentage range of the Beckman instrument used are also determining factors in the degree of accuracy obtainable. The Beckman E-2 model, a null instrument, requires a potentiometric return of the light beam to zero. The technician must adjust

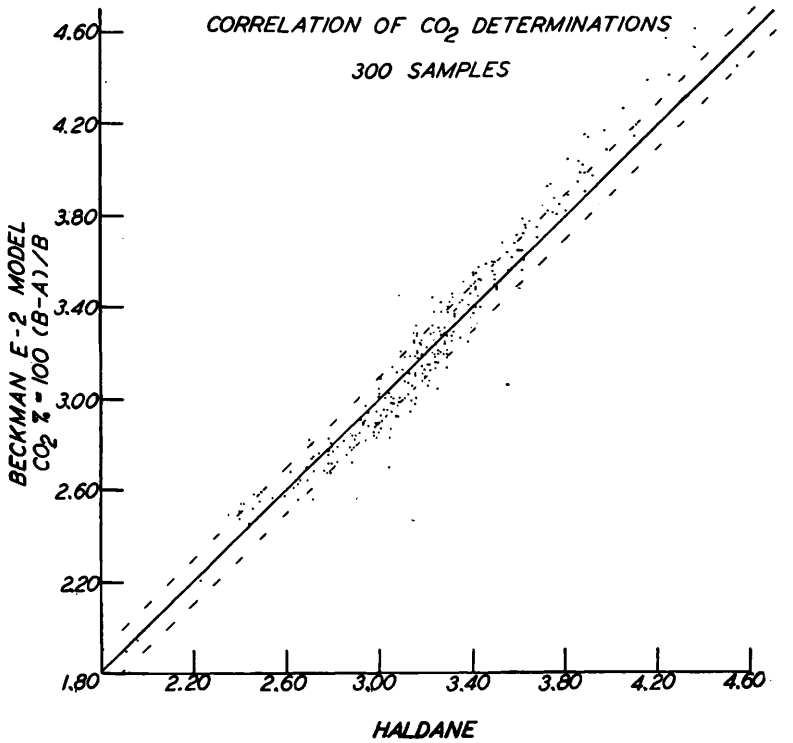

FIG. 3.

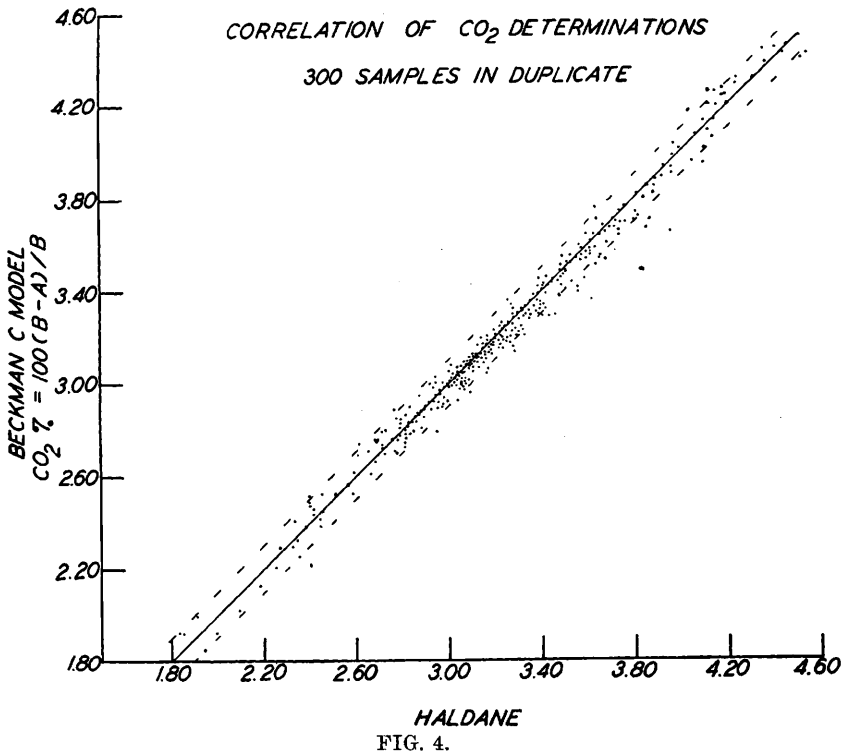

FIG. 4.

the beam to zero and take a reading. This two-step procedure is subject to more error than the simple scale reading of the deflected beam as in the Pauling type C model. Secondly, the E-2 model range of 0-25% is approximately 3½-4 times the range of the C model (110-160 mm Hg). A narrower range on the E-2 model (15-25% or 12-22%) should improve the accuracy. A comparison of results on the E-2 model (Fig. 3) and on the C model (Fig. 4) illustrates this point.

A statistical analysis† of 20 random CO_2 samples, determined by the three instruments was performed using Student's rule of "t". A simple comparison is made in Table II. The Haldane has the best "t" value, indicating that it offers the best accuracy. The C-model Beckman has only a slightly lower "t" value showing that the agreement is excellent. Data on the E-2 model has the lowest "t" value, as one would expect. It is important to note that a determination of the "t" value of the difference between the means points to the fact that all 3 methods are equally valid from a statistical point of view. Here, too, the best agreement is between the Haldane and the C model; the next best agreement is between the Haldane and the E-2 model; while the C and the E-2 differ more widely. This occurs because the E-2 values tend to run on the high side of the Haldane and the C values on low side.

Conclusion. This method offers a simple

TABLE II. Statistical Comparison, 20 Random CO_2 Samples in Duplicate.

	Mean value ± S. E.	t value	t value of difference between means	
Haldane	3.303 ± .90	36.518	.183	.350
Beckman C	3.286 ± .90	36.367	.474	
Beckman E-2	3.351 ± .103	32.525		

† Equations employed in the statistical analysis were: $\alpha = \sqrt{\frac{\Sigma d^2}{n-1}}$; $SEm = \frac{\alpha}{\pm\sqrt{n}}$; $t = \frac{\text{mean}}{SEm}$ and t of difference between means $= \frac{\text{mean}-\text{mean}}{\sqrt{SEm^2 + SEm^2}}$.

quick technic for separate expired air samples. Both CO_2 and O_2 analysis may be completed within 5 minutes as compared to 15-20 minutes by Haldane. Ninety-five per cent of the duplicate CO_2 analyses as determined with the Beckman C model (110-160 mm Hg range) will agree within 0.10% with duplicate Haldane data. A Beckman instrument of a narrower range and/or greater sensitivity would improve this accuracy. In our experience the Beckman C model (110-160 mm Hg range) allows better results than does the more elaborate and expensive E-2 model.

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Activation of Pyruvate Oxidation in Tumor Mitochondria by Diphosphopyridine Nucleotide.* (19089)

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(Introduced by S. P. Reimann.)

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Previous studies from our laboratory have disclosed that intact tumor cells (as exemplified by slices of various transplanted mouse neoplasia) are able to oxidize glucose and fatty acids to completion via the citric acid cycle(1,2). It therefore appeared that previous failures to observe oxidation of pyruvate or oxalacetate in homogenates of tumors(2-6) were due to loss of some labile factor in homogenization rather than to lack of oxidative capacity of the tumor cell.§ In an investigation of this matter it was found that good oxygen uptake by whole homogenates of tumors could be achieved in the presence or absence of substrates if rather high concentrations of diphosphopyridine nucleotide (DPN) were added; some preliminary results of these experiments have already been reported(1). The necessity for DPN in oxidation processes of tumors has been more strikingly demonstrated in experiments with isolated mitochondria, and these are reported in the present communication.

Methods and materials. The tumors used in this study are maintained by subcutaneous implantation in adult mice of the A or C3H strains. When tumors attained a size of 1 cm the animals were sacrificed by decapitation. Mitochondria of normal and neoplastic tissues were obtained by following exactly the procedure of Schneider(7).‡ The washed mitochondria were suspended in isotonic sucrose and kept refrigerated until used. All operations were carried out at $<5°C$. The tissue suspension was added to chilled Warburg vessels containing the ions, factors and substrates designated in Table I. The pyruvate and malate were commercial products. Diphosphopyridine nucleotide was obtained from various commercial sources, and assayed about 40% by the enzymatic procedure of Racker

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† This work will constitute part of a thesis to be presented by C. E. Wenner to the Graduate School of Temple University in partial fulfillment of the requirements for the Ph.D. degree.

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