of 8.6 γ of arginine and 9.8 γ of histidine per ml of plasma while in patient (A.M., No. 6) 18.8 γ of arginine and 6.2 γ of histidine disappeared from the plasma. Thus, while more than twice the amount of arginine disappeared in the latter as compared with the former patient, as regards histidine the situation was reversed. Thus the extent of the relative effect of insulin hypoglycemia on arginine and histidine formation and utilization must be significantly different in these patients. The studies of previous investigators regarding the effect on the level of total amino acids in the blood failed to reveal the complexity of the metabolic processes which require consideration. General speculation as to hydrolysis and synthesis of tissue protein to account for the marked effects of insulin and glucose administration would appear to be a rather crude approach to complex metabolic processes.

In some patients the oral administration of glucose produced as marked a depression in the level of valine and arginine as that seen in insulin hypoglycemic coma. (See patient J.F., No. 10, Table II). However, the effect on the other amino acids was not comparable. This points to some special effects of insulin aside from the supply and utilization of glucose on the metabolism of some of the amino acids. This seems to be especially marked as regards leucine and lysine of the amino acids thus far investigated. In this connection it may be of interest to point out that Steele and his co-workers[23] recently have found that leucine is one of a few amino acids which is not excreted in human urine, either free or combined, except under very special conditions. This occurs in spite of the comparatively high level of leucine in the blood. These findings may point to some special or unique function of leucine in the nitrogen metabolism of man. This will be investigated further.

The possible relation of the amino acids to clinical conditions will be the subject of another paper.

Summary. 1. The effect of insulin hypoglycemic coma and of the oral administration of glucose on the level of various amino acids in the blood have been investigated in 15 mental patients.

2. The level in the blood of all the amino acids, thus far investigated, decreases to a variable extent during insulin hypoglycemia and also after glucose administration.

3. Insulin hypoglycemia produced a more marked effect on the level of blood amino acids than did the administration of glucose. Valine appeared to be an exception in some patients.

4. Leucine and lysine showed the most marked changes of the amino acids thus far investigated.

5. The total amino acids does not reflect the extent of the changes in the blood of the level of the individual amino acids.

6. The possible mechanisms which may account for the changes are discussed.

[23] Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., *J. Nutrition*, 1947, **33**, 209.

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Studies on Cigarette Smoke Irritation. I. Determination of Edema-Producing Properties of Certain Types of Cigarette Tobaccos.

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We described[1] a method for the quantitative evaluation of cigarette smoke irritation, as judged by its edema-producing properties, based on gravimetric determination of the increase in moisture content of the upper

[1] Finnegan, J. K., Fordham, Doris, Larson, P. S., and Haag, H. B., *J. Pharm. and Exp. Therap.*, 1947, **89**, 115.

TABLE I.
Comparative Edema-Producing Properties of Certain Types of Cigarette Tobaccos.

Type of cigarette	No. of observations	Mean of ratio differences*	Mean of % increase in moisture
A. Smyrna (Turkish)	20	0.56	11.82
B. Bright	20	0.96	21.76
C. Maryland	20	1.38	30.76
D. Samsoun (Turkish)	20	1.47	33.79
E. Burley	20	1.71	37.12
F. Blend of A-E	50	1.47	31.14

* Ratios for exposed tissues minus ratios for control tissues.

TABLE II.
Significance of the Differences Observed Between the Edema-Producing Properties of the Types of Cigarette Tobaccos Listed in Table I.

Comparison	P value*	Comparison	P value	Comparison	P value
A vs B	.41	B vs C	.31	C vs E	.39
A vs C	.01	B vs D	.29	C vs F	.76
A vs D	.02	B vs E	.10	D vs E	.70
A vs E	.002	B vs F	.12	D vs F	.98
A vs F	.002	C vs D	.82	E vs F	.44

* Probability values of .05 and less are usually held to indicate statistical significance; .01 and less indicate a high degree of significance.

palpebral conjunctiva of the rabbit eye following its exposure to cigarette smoke. The method is briefly as follows:

Using a smoking machine of the type described by Bradford, Harlan and Hanmer[2] which draws a 35 ml puff once per minute, 3 puffs are directed through a suitable eye cup placed over the widely opened eye of a morphinized rabbit. When one eye only of each rabbit is exposed, the other serving as a control, we have named the technic "Procedure A." Where both eyes of each rabbit are exposed, thereby permitting comparison of 2 cigarettes on the same animal, the technic is called "Procedure B." In both procedures, following exposure to the smoke, a one-hour period is allowed for edema formation. The rabbit is then sacrificed, the upper lid membrane of each eye excised, weighed, dried in an oven to constant weight, and reweighed. From the wet and dry weights the ratio of moisture to dry weight is calculated and, in the case of Procedure A, the per cent increase in water in the exposed membrane over that in the control membrane is determined. In a series of such determinations involving 2 or more groups of cigarettes, the differences in the ratios between groups are evaluated statistically for significance by analysis of variance.

In the present study, we have applied Procedure A to an investigation of a blend of cigarette-tobacco types in an effort to evaluate the role of each in the production of cigarette smoke irritants of the edema-producing type.

Experimental. Six groups of cigarettes were prepared, one containing only Smyrna (Turkish) tobacco, one containing only Samsoun (Turkish) tobacco, one containing only Bright tobaccos, one containing only Maryland tobaccos, one containing only Burley tobaccos, and one containing a blend of all of these tobaccos. All were brought to comparable moisture content (11.5-12.5%) by placing them in atmospheres of suitable humidity. From each group, cigarettes were selected for smoking purposes on the basis of comparable resistance to air flow through them when subjected to a slight constant negative pressure at one end. Fifty of the blended cigarettes and 20 from each of the other groups were tested by Procedure A and the results are recorded in Table I and the significance of differences in Table II.

[2] Bradford, J., Harlan, W., and Hanmer, H. R., *Ind. Eng. Chem.*, 1936, **28**, 836.

Discussion. From Tables I and II it is apparent that the smoke from certain types of cigarette tobaccos tends to be lower in edema-producing irritants than that from other types, and that in a blend of these tobaccos these irritants appear to be additive.

In our original description of the method here used[1] and throughout the presentation of the present study, we have stressed the fact that the only irritants measured by this technic are those that produce edema. That this is not necessarily related to the sensations of subjective irritation experienced by a smoker was strikingly brought out in the present study in the case of the cigarettes made from Burley tobaccos alone. As measured by edema-producing irritants alone this cigarette was not significantly more irritating than the blended cigarette, yet the sensations experienced by the smoker were quite the opposite. Whereas, the average smoker had no difficulty in making a deep inhalation of the smoke from the blended cigarette, a similar inhalation of smoke from the Burley cigarette left him unable to draw a second breath for an uncomfortably long period. It is apparent from this that substances which produce a degree of subjective irritation disproportionately greater than that which might be expected on the basis of their edema-producing properties, may be present in cigarette smoke. Development of a method capable of quantitative measurement of the subjective sensation of irritation to complement the measurement of edema-producing irritants so that a more complete picture may be obtained is greatly to be desired.

Conclusions. 1. Smoke from different types of cigarette tobaccos may differ significantly in edema-producing irritants. 2. Constituents are present in cigarette smoke, from at least certain types of tobaccos, which produce a degree of subjective irritation disproportionately greater than that which might be expected on the basis of their edema-producing properties.

We are indebted to Mrs. Sarah Guerry for technical assistance in performing the reported experiments.

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New Formula for Dilution Curve of Plasma Prothrombin. Normal Standards and Changes in Pathologic Conditions.

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The various one-stage methods for the measurement of prothrombin time used at present, differ little from the procedure originally employed.[1] They are based on the assumption that the clotting time of plasma is dependent solely on its prothrombin content, if an excess of thromboplastin and optimal amounts of calcium are provided; constancy of the rate of conversion of prothrombin to thrombin is assumed. As source of thromboplastin an extract of rabbit brain, or the venom of the Russel viper, are most commonly used.

In all procedures the quantitative comparison of abnormal with normal plasma meets with as yet unresolved difficulties, which have occasioned a variety of methods of assay. The simplest assumption,[2] that the clotting

[1] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. M. Sc.*, 1935, **190**, 501; Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

[2] Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., *Am. J. Clin. Path., Tech. Supp.*, 1940, **4**, 13; Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 595; Govan, C. D., *J. Pediat.*, 1946, **29**, 629.