Table 1. Influence of Actinomycin *D* on the Insulin-induced Stimulation of the Biosynthesis of RNA and Protein in the Isolated Rat Diaphragm

Actinomycin *D* (10 μg/ml.)	0	0	+	+
Insulin (10 μg/ml.)	0	+	0	+
A Biosynthesis of RNA from (8-^{14}C) adenine				
ATP (counts/μg)	201	212	207	280
RNA fraction (counts incorporated/100 mg tissue)	1,006	2,040	26	38
B Biosynthesis of proteins from (U-^{14}C) protein hydrolysate				
Extracellular amino-acid pool (counts/μg)	319	374	327	399
Intracellular amino-acid pool (counts/μg)	66	71	69	72
Proteins (counts/mg)	316	706	395	765

Table 2. Influence of Actinomycin *D* and Puromycin on the Insulin-induced Stimulation of the Labelling of Phosphates in the Isolated Rat Diaphragm

Specific activity (percentage of extracellular or intracellular phosphorus)						
Actinomycin *D* (10 μg/ml.)		0	0	+	+	0	0
Puromycin (500 μg/ml.)		0	0	0	0	+	+
Insulin (10 μg/ml.)		0	+	0	+	0	+
Intracellular inorganic phosphorus	(*E*)	6·9	6·2	7·2	6·7	8·7	10·7
PC*	(*I*)	35·8	44·4	32·7	53·3	31·4	40·5
ATP	(*I*)	44·5	58·5	40·2	68·0	43·4	70·0
ADP	(*I*)	19·7	24·5	19·5	30·2	17·9	21·7
U + G†	(*I*)	24·4	37·9	26·2	51·5	16·7	32·5

(*E*) Percentage of extracellular inorganic phosphorus.
(*I*) Percentage of intracellular inorganic phosphorus.
* Phosphocreatine.
† Sum of uridine and guanosine phosphates.

Table 3. Influence of Puromycin on the Insulin-induced Stimulation of Protein Biosynthesis in Isolated Rat Diaphragm

A Precursor: U-^{14}C protein hydrolysate				
Puromycin (500 μg/ml.)	0	0	+	+
Insulin (10 μg/ml.)	0	+	0	+
Extracellular amino-acid pool (counts/μg)	940	740	545	518
Intracellular amino-acid pool (counts/μg)	115	120	152	131
Proteins (counts/mg)	750	1,371	24	11
B-Precursor: D,L -1^{14}C Valine				
Incubation medium (counts/100 mg)	7,800	8·800	—	8·767
Intracellular medium (counts/100 mg)	6·300	5·745	—	6·352
Proteins (counts/mg)	166	213	—	1

The second set of conclusions concerns the mutual independence of the phenomena which have been tested. This independence is particularly striking so far as protein and RNA biosynthesis are concerned: the complete suppression of the latter has no influence whatsoever on the stimulation of the former; thus it seems difficult to conclude[4] that the biosynthesis of a messenger RNA could even partially mediate the action of insulin on the isolated muscle.

Hence this action seems to consist in a general stimulation of uncorrelated metabolic events; the insulin-induced increase of glucose-uptake may, however, be inhibited by short pre-incubations with *N*-ethylmaleimide[9]. This equally holds for the labelling of energy-rich phosphates[10] and demonstrates that an interaction of insulin with specific —SH groups is a pre-requisite for its effect on the isolated muscle, the precise mechanism of which (for example, cell 'decompartmentation') remain at present a matter of speculation.

D. Eboué-Bonis
A. M. Chambaut
P. Volfin
H. Clauser

Laboratoire de Chimie Biologique,
Faculté des Sciences,
96 Boulevard Raspail,
Paris.

[1] Post, R. L., Morgan, H. E., and Park, C. R., *J. Biol. Chem.*, **236**, 269 (1961).
[2] Kipnis, D. M., and Cori, C. F., *J. Biol. Chem.*, **234**, 171 (1959).
[3] Manchester, K. L., *Biochem. J.*, **81**, 135 (1961).
[4] Wool, I. G., *Biochim. Biophys. Acta*, **68**, 28 (1963).
[5] Clauser, H., Volfin, P., and Eboué-Bonis, D., *Gen. Comp. Endocrinol.*, **2**, 369 (1962).
[6] Jacob, F., and Monod, J., *J. Mol. Biol.*, **3**, 318 (1961).
[7] Mueller, G. C., Gorski, J., and Aizawa, Y., *Proc. U.S. Nat. Acad. Sci.*, **47**, 164 (1961).
[8] Tata, J. R., *Nature*, **197**, 1167 (1963).
[9] Cadenas, E., Kaji, H., Park, C. R., and Rasmussen, H., *J. Biol. Chem.*, **236**, PC 63 (1961).
[10] Volfin, P., Eboué-Bonis, D., Chambaut, A. M., and Clauser, H., *Bull. Soc. Chim. Biol.*, **45**, 458 (1963).

Naphthalenes in Cigarette Smoke

In our work on the composition of the non-methanol volatile neutral fraction of cigarette smoke we have isolated and identified the naphthalenes shown in Table 1.

Table 1

Substance	Yield*
Naphthalene	0·17
2-Methylnaphthalene	0·50
1-Methylnaphthalene	0·50
2,7-Dimethylnaphthalene	0·26
2,6-Dimethylnaphthalene	0·26
1,6-Dimethylnaphthalene	1·30
1,3,6-Trimethylnaphthalene	0·70

* In μg/cigarette (average weight, 1·15 g)

The naphthalenes were isolated from chromatographic fractions of cigarette smoke condensate by precipitation as complexes with *s*-trinitrobenzene. The combined complexes were decomposed and the mixture of naphthalenes obtained was separated by preparative scale gas chromatography. The individual naphthalenes were identified by comparison of ultra-violet and infra-red spectra with those of authentic specimens and by the preparation of solid derivatives. Analytical gas chromatography showed that these were the only naphthalenes present (eluted in the order shown in Table 1) except for a small shoulder appearing on the peak corresponding to 1,6-dimethylnaphthalene. The retention volume of this shoulder corresponded to 1,8-dimethylnaphthalene and/or acenaphthene. Naphthalene, 2-methylnaphthalene, and possibly 1,8-dimethylnaphthalene have been detected already by ultra-violet spectroscopy[1,2]. Acenaphthylene, reported earlier[1], occurs in a later chromatographic fraction of the smoke condensate. The relatively large quantities of dimethyl- and trimethylnaphthalenes have escaped detection by methods used previously, but their presence gives an important lead to the understanding of the mode of formation of polycyclic hydrocarbons in cigarette smoke. The positions of the methyl groups indicate terpenoid precursors of these naphthalenes, and an extension of the suggested mode of thermal decomposition of isoprenoid polyolefines[3] can account fully for their formation.

R. A. W. Johnstone
P. M. Quan

Medical Research Council Unit,
University of Exeter.

[1] Cooper and Lindsey, *Chem. and Indust.*, 1260 (1954); *Brit. J. Cancer*, **9**, 304 (1955). Gilbert and Lindsey, *ibid.*, **10**, 642, 646 (1956).
[2] Bonnet and Nenkomm, *Helv. Chim. Acta*, **39**, 1724 (1956).
[3] Johnstone and Quan, *J. Chem. Soc.*, 2221 (1963).

Boric Acid-induced Heterogeneity of Conalbumin by Starch-gel Electrophoresis

Multiple molecular forms of conalbumin[1], the iron-binding component of avian egg white, have been described by numerous investigators under a variety of experimental conditions. In the original purification of conalbumin, Longsworth *et al.*[2] identified two components, the relative proportions of which were dependent on *p*H. Afterwards, by starch-gel electrophoresis, Lush[3] identified two, and occasionally three, iron-binding proteins in native egg white, as did Williams[4] and Ogden *et al.*[5]. In each of these cases[3–5], the slower migrating of the two components was the more intense; in the latter investigations[4,5] a corresponding heterogeneity was observed in transferrin, the iron-binding protein in the serum of the animal. Thus, in the chicken, as in other species, including man[6], primates[7], cattle[8,9], bison[10], horses[11], and mice[12], a single transferrin allele appears to determine the synthesis of more than one molecular species of transferrin. Ultracentrifugal investigations on purified cattle transferrins indicate that the multiple bovine components do not represent a polymer series[13].

Ogden *et al.*[5] have also described a genetically determined variation in chicken serum transferrin which is