those found for *L*-929, an established fibroblast line with a long culture history[14] and some neoplastic potential[15]. In other experiments an SV_{40} transformed line derived from a human diploid fibroblast strain[16] was similarly found to be unresponsive to ascorbate supplementation and to have a lower rate of collagen synthesis than the parent strain.

Table 1. COLLAGEN SYNTHESIS BY FIBROBLAST LINES AND THEIR VIRAL TRANSFORMANTS

	HyPro/Pro (%)		$\Delta C/\Delta P$ (%)	
	low ascorbate	ascorbate supplemented	low ascorbate	ascorbate supplemented
3*T*6	9·47	15·4	3·5	6·4
3*T*3	1·60	17·2	0·55	7·2
SV-3*T*3-5	5·35	—	1·93	—
SV-3*T*3-10 clone 1	2·31	13·7	0·80	5·5
SV-3*T*3-5 clone 3	1·88	9·9	0·65	3·8
SV-*PY*-3*T*3-11 clone 11	4·65	4·24	1·66	1·51
L-929	4·56	4·65	1·60	1·66

Collagen synthesis in the transformed cell lines therefore differs from that in the parent cells in two respects: (1) When tested under conditions that allow the maximal rate of collagen formation (adequate ascorbate supplementation) the viral transformants produce less collagen per unit of protein synthesized than the parent line. A second differentiated function, hyaluronate synthesis, also is reduced in transformed 3*T*3 cells[17]. (2) Collagen synthesis in the transformed cells is less dependent on exogenously supplied ascorbate. The first result is consistent with the commonly observed loss of differentiated function occurring in neoplastic cells; the second with an increased metabolic autonomy.

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Incorporation of ^{35}S-methionine into Insulin

IN our investigations[1] of the biosynthesis of the *A* and *B* chains of insulin in rats by means of labelled amino-acids, we studied the incorporation of ^{35}S-methionine into that protein, the presence of which has not yet been experimentally demonstrated in insulin. There are some indications in the literature that the amino-acid composition of insulin in rats differs somewhat from that in animals of other species[2–4].

We carried out an investigation *in vivo* of the incorporation of ^{35}S-methionine into insulin of rats. The labelled amino-acid was injected intraperitoneally into the rat (1·2 μc./1·0 g body-weight; spec. act., 530 μc./mg). The animals were killed at various times after the injection. In some experiments insulin was isolated by Petting's method[5]. To purify the admixtures, the isolated insulin was reprecipitated many times at its isoelectric point. The radioactivity of the 'purified' insulin was determined, and results obtained are shown in Table 1.

Table 1. INCORPORATION OF ^{35}S-METHIONINE INTO RAT INSULIN AT VARIOUS TIMES AFTER INJECTION OF LABELLED AMINO-ACID (c.p.m./mg)

Time (min)	40	80	120
Radioactivity (c.)	240	540	680

Due to the possibility that the radioactivity found in insulin after injection of ^{35}S-methionine could be the result of a transformation of the labelled amino-acid into cysteine, followed by incorporation into a molecule of insulin, we determined the amino-acid residues of insulin to which activity belonged. For this purpose, insulin labelled *in vivo* with ^{35}S-methionine was oxidized by Sanger's method[6]; the chains were separated by high-voltage electrophoresis (1·0 mg oxidized insulin was transferred to the electrophoregram, and electrophoresis was carried out in phosphate buffer at *p*H 8·6 and 1,000 V). After eluting the insulin chains with dilute alkali (followed by evaporation under vacuum) the complete hydrolysate of each of the chains was radiochromatographed. The chromatograms of hydrolysates of the *A* and *B* chains of insulin showed radioactivity in the methionine and cysteine positions. In chain *A* 70 per cent of all activity was found in methionine and 30 per cent in cysteine, while in chain *B* the radioactivity of methionine and cysteine was the same (Table 2).

Table 2. DISTRIBUTION OF RADIOACTIVITY BETWEEN METHIONINE AND CYSTEINE RESIDUES IN INSULIN AND ITS *A* AND *B* CHAINS AFTER INJECTION OF LABELLED AMINO-ACID (c.p.m./mg)

Mode of isolation	Amino-acid residues	Insulin	Chain *A*	Chain *B*
Isoelectrical precipitation	^{35}S-methionine	540	350	509
	^{35}S-cysteine	498	150	417
Immunological	^{35}S-methionine	72	—	—
	^{35}S-cysteine	60	—	—

Thus, the data obtained show that ^{35}S-methionine is incorporated into both chains as such, and in addition that its radioactive sulphur resides in the cysteine residues of insulin.

The incorporation of ^{35}S-methionine into insulin in the process of biosynthesis of this protein was also confirmed by isolating insulin immunologically[7]. Complete acid hydrolysis and radiochromatography of the antigen–antibody complex revealed that the radioactivity resided in the methionine and cysteine positions (Table 2). It should be noted that in this experiment 55 per cent of the radioactivity of the original methionine was found incorporated in the cysteine residues of the insulin chains. This finding is in close agreement with the data obtained when insulin was isolated isoelectrically.

The present data indicate that ^{35}S-methionine is incorporated into rat insulin and that it is, in fact, a constituent amino-acid of this protein.

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