the intensity of the signal produced by the interference of these two beams in a third part of the crystal. They find that the relative phase of the two interfering beams changes as elementary quantum mechanics predicts it should when the apparatus is rotated so as to alter the relative inclination of the two beams to the horizontal.

Of course one is not very surprised that the result of a low-energy experiment of this type is accurately predicted by conventional quantum theory. It is unfortunate that weak gravitational fields should be the only ones accessible to experiment since recent remarkable results due to Hawking and Beckenstein indicate profound interrelations between quantum theory, gravitation and thermodynamics.

Hawking discovered that, in the case of a realistic black hole formed by stellar collapse, effects to do with the quantum fluctuations of the vacuum cause real particles to be produced and emitted to infinity as a constant flux. What is really astonishing about Hawking's result is its form. The outcome of his highly sophisticated analysis is that black holes should radiate photons and other particles precisely as if they were black bodies of definite temperature. The temperature is inversely proportional to the mass of the hole.

Surprisingly, this result is essentially that suggested earlier by Beckenstein (*Phys Rev.*, **D7**, 2333; 1973) from an entirely different, very classical argument: Take a large, brassy Victorian bedstead and consider it carefully for a moment. You observe four legs, many knobs and springs. Indeed if you are a connoisseur of such things you might well guess that it was manufactured in Bristol in 1864. Toss this same bedstead into a black hole and all this information is suddenly lost to you. The only differences that you observe in the hole are small changes in its mass and angular momentum. But of course a loss of information implies an increase in entropy, an effect which one can quantify. In this way Beckenstein was able to put a value on the entropy of a black hole, which turned out to be proportional to its surface area. From this followed in a formal way a value for its thermodynamic temperature. What Hawking showed us was that this formal temperature is perfectly real.

Thes two results fit beautifully with work by Bardeen, Carter and Hawking (see for example Carter's contribution to *Black Holes,* edit. by DeWitt and DeWitt; Gordon and Breach, 1973) who showed that changes in the configuration of a black hole are constrained by a series of laws analogous to those of thermodynamics. This similarity was at first thought entirely formal with the inverse of the black hole mass having the role of temperature and the area of the black hole behaving like entropy. Now in the light of the work by Hawking and Beckenstein we see that there is a complete identification of black hole parameters and thermodynamic variables.

It is very encouraging that a field as young as is the study of quantised particles in gravitational fields should already have produced a much needed piece of the jig-saw of physics. A host of cosmological problems seem to require for their solution a better understanding of these quantum processes. Perhaps with the solution of these other problems we will again obtain important and unexpected insights into the nature of things.

Spots before the eyes

from E. G. Richards

A RECENTLY published two-dimensional electropherogram shows the resolution of more than 1,100 proteins from *E. coli.* This spectacular result is to be found in a paper by O'Farrell (*J. biol. Chem.*, **250**, 4007; 1975).

Two-dimensional electrophoresis involves the separation of a mixture along the first dimension according to some molecular property of the components; this is followed by movement of the components in a perpendicular direction resulting in separation according to another property. Most attempts to apply such methods to mixtures of proteins have suffered from correlations between the two chosen properties resulting in a tendency of the components to cluster round a diagonal in the final pattern.

O'Farrell has played the simple trick of separating according to isoelectric point by isoelectric focusing in the first dimension followed by a separation according to molecular weight by SDS-polyacrylamide electrophoresis in the second. The isoelectric point and molecular weight show virtually no correlation.

To summarise his method, the separation in the first dimension is conducted in a 2¼% cylindrical polyacrylamide gel containing 9 M urea and ampholines to produce a *p*H gradient between *p*H 5 and 7. For the second dimension a 0.8 mm thick flat bed gel of polyacrylamide with an exponential concentration gradient and containing SDS is used. The sample to be applied is first treated with nucleases to remove RNA and DNA which otherwise produce streaking effects and then the proteins are solubilised and denatured in 9 M urea before application to the first dimension. After the first separation the isoelectric gel is advantageously equilibrated by shaking in SDS solution before incorporation into the flat bed gel. Although this equilibration leads to some loss of proteins and some impairment of the resolution, it further reduces streaking.

The authors claim that about 70 bands can be resolved in the first isoelectric separation, and up to 100 in the second SDS dimension. Thus a maximum of 7,000 proteins could in principle be resolved though this figure may be reduced somewhat in practice for a variety of technical reasons. Nevertheless the authors have counted more than 1,100 spots obtained from the application of whole *E. coli* extracts. A rough calculation which assumes a chromosome of 4 million base pairs and a thousand of these to each protein gives an upper limit of 4,000 different proteins in *E. coli.*

Two detection systems were utilised: staining with Coomassie blue and autoradiography with the use of ^{14}C labelled proteins. The first method can detect 0.01 μg of protein in a spot; the second, in a twenty day exposure, can detect 1 c.p.m. which could correspond to a protein which existed in but one copy per cell at realistic, albeit high, specific activities. However these detection limits are by no means the whole story since the picture is complicated when the total load of protein is raised to enable minor components to be detected. In the first place the total protein load must be kept low to avoid streaking effects and other distortions of the spot shape; in the second the size of a spot was found to increase when the amount of protein in it increased. This means that minor components located close to abundant proteins would be hidden and remain undetected.

The authors claim that replicate runs are highly reproducible provided the conditions are carefully standardised and ampholines from the same batch are used. The comparison of two electropherograms is facilitated by the characteristic patterns of spots produced.

If the author's recipes are not carefully adhered to, certain artefacts may be introduced such as streaking and multiple spots due to charge heterogeneity and solubility problems.

It would thus sem that a new tool of considerable power has arrived and it will be interesting to see what use is made of it. O'Farrell himself says that the method as it stands does not work for basic proteins such as ribosomal proteins and histones but one may guess that there are no insuperable problems in adapting it for such mixtures. Maybe the characteristic patterns of spots will also commend themselves to taxonomists.