These values are similar to what might be expected for murine mAb from murine hybridomas when cultured in vitro. The purified rabbit mAbs have been used for successful development of ELISA systems for GAS detection.

Karyotyping of one RMH (B52) was performed to ascertain the number of chromosomes present at various times after fusion. By 64 days after fusion, the mean chromosome number of this line ($2n = 69$) had stabilized to approximately the same as the parent SP2/0 cells ($2n = 65$), with a coefficient of variation (5.6%) less than half that of the SP2/0 cells (13.3%). The karyotypes of four other RMH lines were also examined 3 months after fusion and found to contain a similar number of chromosomes. The nuclei of these RMH lines appear to contain three to five rabbit metacentric chromosomes, in addition to their complement of mouse acrocentric chromosomes (Fig. 1).

We have developed optimized procedures for producing large numbers of stable RMHs that secrete complete rabbit Ig molecules with excellent specificity to the GAS immunogen used. These procedures include the use of the SP2/0-Ag14 myeloma line as fusion partner, culture of RMHs after fusion in the presence of NRS and PEC feeder cells, cloning of RMHs in medium containing NRS, and once stabilized, gradual adaptation of monoclonal RMH lines to grow in medium containing FCS rather than NRS. The ability to use spleen cells from specificity hyperimmunized rabbits for generation of large numbers of stable RMH lines that secrete rabbit mAb against these immunogens should allow production of new mAb species that, to date, could not be produced.

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11. In the ELISA we used PVC microtiter plates (Dynatech Laboratories) coated with an optimized dilution (in 0.1*M* carbonate-bicarbonate buffer, *p*H 9.6) of nitrous acid extract (*10*) from *Streptococcus* cells of groups A, B, C, D, F, or G (ATCC 19615, E13813, 12388, 19433, 12392, or 12394; recommended by ATCC as being type strains antigenically representative of groups A, B, C, D, F, and G, respectively). Positive cultures were detected with an optimized dilution of urease-conjugated sheep antibody to rabbit IgG (H and L chain specific) and urease substrate. They were confirmed to be secreting rabbit Ig by differential reactivity in ELISA using this conjugate and optimally diluted urease conjugated rabbit antibody to mouse $F(ab')_2$ (Ureiase system, Allelix Diagnostics). The specificities of both these conjugates for rabbit and mouse Ig in the ELISA was established by using titrations of GAS-reactive rabbit and mouse antisera. Control wells were used in which DMEM supplemented with FCS or NRS was substituted for RMH culture supernatant. The absorbance matrix at 590 nm of ELISA plate wells was read on a Multiscan ELISA reader (Flow Laboratories, Inglewood, CA) calibrated on appropriate control wells.
12. Fusion of spleen cells to plasmacytoma cells in the ratio of 5 to 1 was mediated with 43% w/v polyethylene glycol 4000 (Merck, Rahway, NJ).
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16. The anti-rabbit IgG immunoadsorbent was prepared from affinity-purified goat antibody to rabbit IgG (H + L) (Jackson Immunoresearch Laboratories) coupled to CNBr Sepharose 4B (Pharmacia Canada Inc., Dorval, Quebec, Canada) at 2 mg of protein per gram of gel, and poured into a column on top of Sephadex G-25 (Pharmacia Canada Inc.) (*19*). The specificity of this immunoadsorbent column for rabbit Ig was shown by passing culture supernatant and ascitic fluid containing murine mAb against GAS through it, and eluting with 2*M* sodium thiocyanate under conditions identical to those used for RMH culture supernatants. ELISAs on these control eluates showed that they contained neither mouse nor rabbit antibody against GAS, proving that the immunoadsorbent column bound only rabbit Ig.
17. Feeder cells were prepared from normal BALB/c mice by the methods of S. Fuller, M. Takahashi, and J. G. R. Hurrell [in *Current Protocols in Molecular Biology*, F. M. Ausubel *et al.*, Eds. (Wiley Interscience, New York, 1987), chap. 11]. Splenocytes and thymocytes (mixed in a 1:1 ratio) were seeded at 10^6 cells per well, whereas peritoneal exudate cells were seeded at 10^4 cells per well.
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A New Specimen of *Archaeopteryx*

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A new specimen of the primordial bird *Archaeopteryx* is reported from the Upper Jurassic Solnhofen Limestone of Bavaria. This "Solnhofen specimen" is the largest of now six skeletal specimens and shows close similarities with the London specimen. It is therefore assigned to *Archaeopteryx lithographica* Meyer. Clear impressions of the feather shafts of the left wing are preserved.

A NEW, WELL-PRESERVED SPECIMEN of *Archaeopteryx* from the Upper Jurassic Solnhofen Limestone (Lower Tithonian) was discovered in a private collection in Solnhofen, West Germany, in November 1987. It is the sixth skeletal specimen of this famous fossil after the London, Berlin, Maxberg, Haarlem, and Eichstätt specimens (*1*) [unless one follows the suggestion of Howgate (*2*) to separate the smallest one, the Eichstätt specimen, as a distinct genus]. The new example is the largest of all, about 10% larger than the London specimen. The new specimen is housed and exhibited in the Bürgermeister-Müller-Museum in Solnhofen, and is therefore announced here as the "Solnhofen specimen" of *Archaeopteryx* (Fig. 1).

Exact locality data are not available. According to the collector, an amateur, the specimen was found many years ago and no data were kept. After preliminary preparation, carried out only recently, he took it for a specimen of the small theropod dinosaur *Compsognathus*, rather than of *Archaeopteryx*. Although the quarry site is no longer known, it must have been located in the Eichstätt area where the Berlin and Eichstätt specimens were found in 1877 and 1951, respectively (*3*).

The skeleton is quite complete and in almost natural articulation. It is preserved on a slab (52 by 39 cm) of Solnhofen limestone of the so called "Fäule" facies, a soft marly limestone with a content of 10 to 20% clay minerals (*4*). Preservation of the dark brown bone is generally good, and detailed preparations by both mechanical

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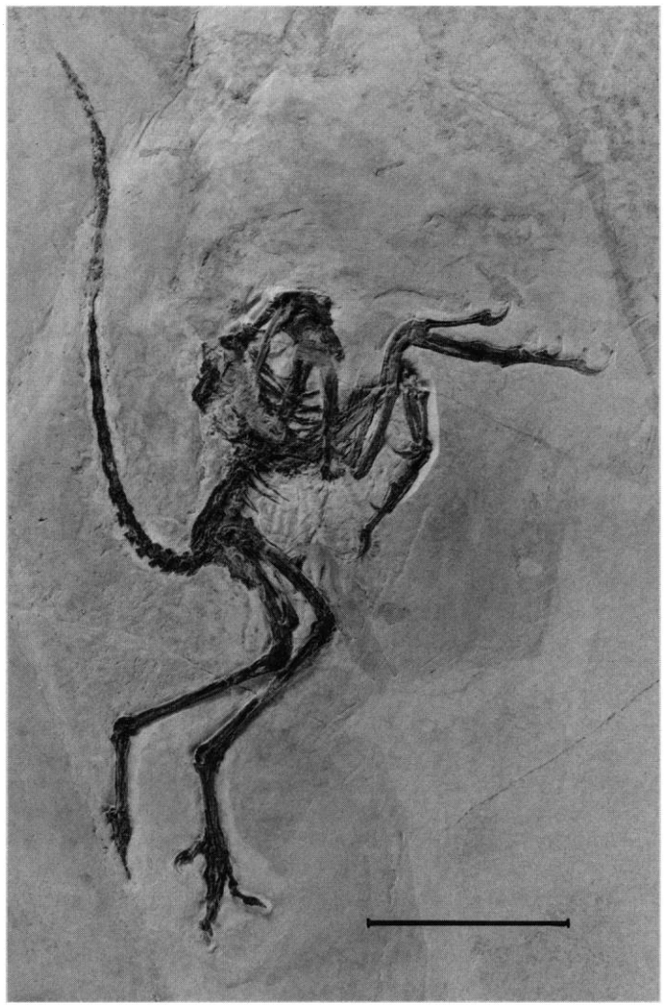

Fig. 1. *Archaeopteryx lithographica* Meyer, the new specimen ("Solnhofen specimen"), from the Solnhofen Limestone, Upper Jurassic, of Bavaria, West Germany. The distal end of the tail has been restored longer than is possible. Scale bar, 10 cm.

Fig. 2. The "Solnhofen specimen" of *Archaeopteryx*, drawn from Fig. 1. The remains of the skull are set off in black. The tail has been restored to its probable length. Impressions of the shafts of the left wing feathers are indicated by dotted lines.

tools and air abrasives carried out at the Bavarian State Collection of Palaeontology and Historical Geology in Munich revealed such delicate structures as the extremely thin and pointed horny sheaths of the finger claws.

Originally the slab was broken in several pieces. Along the cracks bone material was lost. At first glance it seemed that the skull was missing. Only after extensive preparation it turned out that some skull elements (the snout with teeth) were present. However, it is most disappointing that critical parts of the skull were lost during or after excavation. The four preserved premaxillary teeth are similar to the teeth of the London and the Berlin specimens.

The tail is preserved as far as the 15th caudal vertebra. By comparison with the other specimens, it is estimated that eight distal caudals are missing. [On the slab the tail has been restored by the collector to a much greater length (Fig. 1).] In contrast to the specimens of London, Berlin, and Eichstätt, the tail of the new specimen is bent sharply upward immediately behind the pelvis. Also the neck seems to have been bent strongly backward and to the right side, so much so that the skull lies across the dorsal vertebral series (Fig. 2). In front of the shoulder girdle a fragment of a curved bone pressed against the coracoid may represent the furcula, a typical avian structure. In both hands the third finger crosses the second one, exactly as preserved in the Berlin, Maxberg, and Eichstätt specimens (*5*). The third finger consists of four phalanges giving further support for a theropod-like phalangeal formula of the manus in *Archaeopteryx* with two, three, and four phalanges in the first, second, and third digits, rather than two, three, and three as suggested by others (*6*). Both pubes are fused distally in a symphysis forming the typical "pubic foot" as documented in the London, Berlin, and Eichstätt specimens.

The osteological characters of this new specimen show close similarities to the London specimen, especially. This includes the peculiar tooth architecture and the relative proportions of the limbs (Table 1). The new specimen can thus be assigned to *Archaeopteryx lithographica* Meyer. It is about 10% larger than the London specimen, as mentioned, and 30% larger than the Berlin specimen, but is double the size of the Eichstätt specimen. The latter also differs in the hind limb proportions, which are relatively longer than in the other individuals (*3*). Whether the Solnhofen specimen of *Archaeopteryx* adds new arguments in favor or against a generic or specific separation of the small, and possibly juvenile, Eichstätt specimen as *Jurapteryx recurva* (Howgate, 1984) (*2*) will be discussed elsewhere together with a detailed description of the new specimen (*7*).

Because Hoyle and Wickramasinghe (*8*) have speculated that the feather imprints found in the London and Berlin specimens

Table 1. Measurements of the new Solnhofen specimen of *Archaeopteryx lithographica*.

Skeletal element	Length (mm)
Tail, restored	240*
Coracoid	24.5
Humerus	83.0
Ulna	72*
Radius	69*
Digit I	45.9
Digit II	65.4
Digit III	43.6
Hand length	105*
Ilium	38*
Ischium	24.5
Pubis	59.3
Femur	70*
Tibia	89.5
Fibula	82.4
Foot length	95*

*Approximate measurements.

of *Archaeopteryx* are forgeries, one is eager to see whether the Solnhofen specimen shows traces of feathers. Under low-angle illumination there are distinct parallel impressions originating from the lower arm of the left wing skeleton. These features (Fig. 2) are interpreted as imprints of feather shafts. Furthermore, impressions subparallel to the left fingers may mark the outline of the wing. No traces of tail feathers, as in the London, Berlin, and Eichstätt specimens, are visible, however. Because a question of a forgery in the new specimen does not arise, the impressions of the wing feathers are direct evidence that *Archaeopteryx* had feathers (*9*).

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Neuroregulatory and Neuropathological Actions of the Ether-Phospholipid Platelet-Activating Factor

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Platelet-activating factor (PAF) is a naturally occurring phospholipid that serves as a critical mediator in diverse biological and pathophysiological processes. In this study of the interactions of PAF with neuronal cells, it was found that PAF increased the intracellular levels of free calcium ions in cells of the clones NG108-15 and PC12. The increase was dependent on extracellular calcium and was inhibited by the antagonistic PAF analog CV-3988 and by the calcium-influx blockers prenylamine and diltiazem. A functional consequence of this interaction was revealed by measuring a PAF-elicited, Ca^{2+}-dependent secretion of adenosine triphosphate from PC12 cells. Exposure of NG108-15 cells for 3 to 4 days to low concentrations of PAF induced neuronal differentiation; higher concentrations were neurotoxic. Thus, by influencing Ca^{2+} fluxes, PAF may play a physiological role in neuronal development and a pathophysiological role in the degeneration that occurs when neurons are exposed to circulatory factors as a result of trauma, stroke, or spinal cord injury.

PLATELET-ACTIVATING FACTOR (PAF, PAF-acether) is a naturally occurring alkyl-ether phospholipid: 1-*O*-alkyl, 2-acetyl-*sn*-glyceryl-3 phosphorylcholine (*1*). In addition to being a potent activator of platelets, PAF serves as an extracellular mediator in the process of inflammation, has antihypertensive activity, induces smooth muscle contraction, increases vascular permeability, and has been implicated in several pathological processes involving various tissues and cell types (*2*). Brain tissue contains relatively high levels of enzymes for the synthesis and metabolism of alkyl-ether phospholipids (*3*), and it has been suggested that PAF may play a role in the regulation of neuronal function (*4*). We have now examined directly the interactions of PAF with neuronal cells of the hybrid cholinergic clone NG108-15 and the adrenergic clone PC12 (*5*). By means of the bioluminescent calcium probe aequorin, it was found that in these cells PAF induces an increase in intracellular free Ca^{2+}. This increase is associated with stimulated uptake of Ca^{2+} from the extracellular environment and increased vesicular release.

Neural cells of the hybrid clone NG108-15 were grown in culture in a chemically defined medium (*6*). Within 24 to 48 hours after the addition of 50 n*M* PAF to the medium, the growth of the cells was arrested followed by morphological differentiation with neurite extension (Fig. 1A). This differentiating effect of PAF was concentration and time dependent (*7*): maximal effects were observed at 2.5 μ*M* PAF after 3 to 4 days of exposure. Addition of higher concentrations of PAF (3.5 to 10 μ*M*) produced cytotoxic effects. With increasing exposure time to these PAF concentrations the cells gradually rounded up and then lost viability (Fig. 1B). Thus, low concentrations of PAF may play a role in neuronal development, whereas higher PAF concentrations cause neurodegeneration.

Elevated concentrations of intracellular free Ca^{2+} are known to induce morphological changes in neurons, whereas a pathophysiological increase in intracellular calcium can be neurotoxic. To examine whether the dual effects (neurite extension and neurotoxicity) of increasing PAF concentrations on NG108-15 cells are mediated by this mechanism, we used cells loaded with aequorin. The addition of PAF resulted in a marked increase in the level of intracellular Ca^{2+} as measured by aequorin (Fig. 2A, left side); from a basal level of <1 μ*M* it increased to 7 μ*M* Ca^{2+} after stimulation with 4.5 μ*M* PAF. When 3 m*M* EDTA or 1.8 m*M* EGTA were added to the medium, the aequorin signal induced by PAF was completely blocked (Fig. 2A, right side). These results suggest that the PAF-induced increase in intraneuronal Ca^{2+} involves uptake of extracellular Ca^{2+}, a suggestion supported by the effects of inhibitors of Ca^{2+} influx, discussed below.

We also tested the effects of PAF on an adrenergic cell line, PC12 cells. The results were similar to those obtained with the cholinergic NG108-15 cells (Fig. 2, B and C). Although the cells showed responsiveness to nanomolar concentrations of PAF (see Fig. 2B, inset), the aequorin signal could be best quantitated when we used micromolar PAF concentrations. A hyper-

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